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## A critical review to identify data gaps and improve risk assessment of bisphenol A alternatives for human health

Sakina Mhaouty-Kodja<sup>a\*</sup>, Daniel Zalko<sup>b\*</sup>, Sabrina Tait<sup>c\*</sup>, Emanuela Testai<sup>d\*</sup> , Catherine Viguié<sup>b\*</sup>, Emanuela Corsini<sup>e</sup>, Nathalie Grova<sup>f</sup>, Franca Maria Buratti<sup>d</sup>, Nicolas J. Cabaton<sup>b</sup>, Lucia Coppola<sup>c</sup>, Antonio De la Vieja<sup>g</sup>, Maria Dusinska<sup>h</sup>, Naouale El Yamani<sup>h</sup>, Valentina Galbiati<sup>e</sup>, Patricia Iglesias-Hernández<sup>g</sup>, Yvonne Kohl<sup>i</sup>, Ambra Maddalon<sup>e</sup>, Francesca Marcon<sup>d</sup>, Lydie Naulé<sup>a</sup>, Elise Rundén-Pran<sup>h</sup>, Francesca Salani<sup>d</sup>, Nicoletta Santori<sup>d</sup>, Mónica Torres-Ruiz<sup>j</sup>, Jonathan D. Turner<sup>f</sup>, Ondrej Adamovsky<sup>k</sup>, Kiara Aiello-Holden<sup>l</sup>, Hubert Dirven<sup>m</sup>, Henriqueta Louro<sup>n,o</sup> and Maria João Silva<sup>n,o</sup>

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### ABSTRACT

Bisphenol A (BPA), a synthetic chemical widely used in the production of polycarbonate plastic and epoxy resins, has been associated with a variety of adverse effects in humans including metabolic, immunological, reproductive, and neurodevelopmental effects, raising concern about its health impact. In the EU, it has been classified as toxic to reproduction and as an endocrine disruptor and was thus included in the candidate list of substances of very high concern (SVHC). On this basis, its use has been banned or restricted in some products. As a consequence, industries turned to bisphenol alternatives, such as bisphenol S (BPS) and bisphenol F (BPF), which are now found in various consumer products, as well as in human matrices at a global scale. However, due to their toxicity, these two bisphenols are in the process of being regulated. Other BPA alternatives, whose potential toxicity remains largely unknown due to a knowledge gap, have also started to be used in manufacturing processes. The gradual restriction of the use of BPA underscores the importance of understanding the potential risks associated with its alternatives to avoid regrettable substitutions. This review aims to summarize the current knowledge on the potential hazards related to BPA alternatives prioritized by European Regulatory Agencies based on their regulatory relevance and selected to be studied under the European Partnership for the Assessment of Risks from Chemicals (PARC): BPE, BPAP, BPP, BPZ, BPS-MAE, and TCBPA. The focus is on data related to toxicokinetic, endocrine disruption, immunotoxicity, developmental neurotoxicity, and genotoxicity/carcinogenicity, which were considered the most relevant endpoints to assess the hazard related to those substances. The goal here is to identify the data gaps in BPA alternatives toxicology and hence formulate the future directions that will be taken in the frame of the PARC project, which seeks also to enhance chemical risk assessment methodologies using new approach methodologies (NAMs).

**Abbreviations:** ADME: absorption, distribution, metabolism, and excretion; AhR: aryl hydrocarbon receptor; AR: androgen receptor; AUC: area under the curve; BADGE: bisphenol A diglycidyl ether; BBB: blood–brain-barrier; BCRP: breast cancer resistance protein; BDNF: brain-derived neurotrophic factor; BFDGE: bisphenol F diglycidyl-ether; BPA: bisphenol A; BPA-G: BPA mono-glucuronide; BPAF: bisphenol AF; BPAP: bisphenol AP; BPB: bisphenol B; BPBP: bisphenol BP; BPC: bisphenol C; BPE: bisphenol E; BPF: bisphenol F; BPFDE: bisphenol F diglycidylether; BPFL: bisphenol FL; BPM: bisphenol M; BPP: bisphenol

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### KEYWORDS

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P; BPPH: bisphenol PH; BPS: bisphenol S; BPS-G: BPS-glucuronide; BPS-IP: BPS-isopropyl; BPS-MAE: bisphenol S 4-allyl ether; BPZ: bisphenol Z; CAR: constitutive androstane receptor; CGCs: cerebellar granule cells; CLP: Classification, Labelling, and Packaging; CHO: Chinese hamster ovary; COMT: catechol O methyl transferase; CTA: cell transformation assay; CYP: cytochrome P450; DHEA: dehydroepiandrosterone; DHT: 5 $\alpha$ -dihydrotestosterone; DIO: iodothyronine deiodinase; DNT: developmental neurotoxicity; E2: 17 $\beta$ -estradiol; EC: effective concentration; ECHA: European Chemicals Agency; ED: endocrine disrupting; EDC: endocrine disrupting chemical; EFSA: European Food Safety Authority; ER: estrogen receptor; ERR: estrogen-related receptor; FOXE1: forkhead box protein E1; FXR: farnesoid X receptor;  $\gamma$ H2AX: histone family member X; GIVIMP: Guidance Document on Good *In Vitro* Method Practices; GM-CSF: granulocyte-macrophage colony-stimulating factor; GPER: G protein-coupled estrogen receptor; GR: glucocorticoid receptor; hCG: human chorionic gonadotropin; HED: human equivalent dose; hESCs: human embryonic stem cells; HFR: halogenated flame retardants; HIM: human intestine microsomes; HLC: human liver cytosol; HLM: human liver microsomes; HSA: human serum albumin; HSD: hydroxysteroid dehydrogenase; HHTK: high throughput toxicokinetics; IFN- $\gamma$ : interferon gamma; IL: interleukin; IL1rap11: interleukin 1 receptor accessory protein like 1; NR: nuclear receptor; LXR: liver X receptor; MCT8: monocarboxylate transporter 8; MDC: metabolism disrupting chemical; MR: mineralocorticoid receptor; NAM: new approach methodology; Ncam2: neural cell adhesion molecule; NCoR: nuclear receptor corepressor; NF- $\kappa$ B: nuclear factor-kappaB; NGRA: new generation risk assessment; NIS: sodium/iodide symporter; NKX2.1: NK homeobox protein 1; NK: natural killer; NSCs: neural stem cells; OECD: Organisation for Economic Cooperation and Development; PARC: Partnership for the Assessment of Risks from Chemicals; PAX8: paired box 8; PBK: physiological based kinetic; PPAR: peroxisome proliferator-activated receptor; PR: progesterone receptor; PXR: pregnane X receptor; QSAR: quantitative structure–activity relationship; QIVIVE: quantitative *in vitro* to *in vivo* extrapolation; RAR: retinoic acid receptor; REACH: European regulation on the Registration, Evaluation, Authorisation and restriction of Chemicals; ROR: RAR-related orphan receptor; ROS: reactive oxygen species; RXR: retinoid X receptor; SCENIHR: Scientific Committee on Emerging and Newly Identified Health Risks; SERM: selective estrogen receptor modulator; SHE: Syrian hamster embryo; SMRT: silencing mediator for retinoid and thyroid-hormone receptor; SNAP: synaptosomal-associated protein; StAR: steroidogenic acute regulatory protein; SULT: sulfotransferase; SVHC: substance of very high concern; T3: triiodothyronine; T4: thyroxine; TBBPA: tetra-bromo bisphenol A; TBG: thyroxine-binding globulin; TCBPA: tetra-chloro bisphenol A; TDI: tolerable daily intake; TG: thyroglobulin; TGF: transforming growth factor; Th: T helper; TH: thyroid hormone; THR: thyroid hormone receptor; TK: toxicokinetics; TMBPA: tetra-methyl BPA; TNF- $\alpha$ : tumor necrosis factor alpha; TPO: thyroperoxidase; TSH: thyroid-stimulating hormone; TSHR: thyroid-stimulating hormone receptor; TTR: transthyretin; UGT: UDP glucuronosyltransferase; VDR: vitamin D receptor; WoE: weight of evidence; WHO: World Health Organization; XME: xenobiotic metabolizing enzymes

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## 1. Introduction

Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl) propane) is a synthetic chemical used to produce polycarbonate plastic and epoxy resins. Polycarbonates are used as food contact material and epoxy resins in the manufacture of inner coatings for beverage and food cans, but also in the production of many consumer products, water-pipes, electronic equipment, as well as dental composites and other medical devices (Michałowicz 2014). BPA was also used in the EU in thermal papers such as cash register receipts until 2022, when a new EU regulation entered into force drastically limiting the BPA final content (0.02% as maximum level) (European Commission 2016). Human exposure to BPA is widespread and measurable levels have been detected in many matrices (Vandenberg et al. 2010; Michałowicz 2014). Although BPA has a low acute toxicity, prolonged ingestion has been associated with several adverse effects in animal studies (Jun et al. 2021). In 2016, BPA was classified in Europe as toxic to reproduction (category 1B “may impair fertility”), according to the European Commission Regulation No. 1272/2008, on the Classification, Labelling, and Packaging (CLP) of substances and mixtures. It was also identified as a substance of very high concern (SVHC) in the framework of the European REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (ECHA 2017), meaning that the substance may only be marketed and used for specific authorized purposes. A number of EFSA (European Food Safety Authority) opinions have been published since 2006 related to its presence in food contact materials, the last one being adopted in 2023 (EFSA 2023). In addition, a SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) opinion evaluated potential risks associated to leakage from medical devices (SCENIHR 2015).

BPA has been described to induce a number of adverse health effects with the attention often focused on effects mediated by its endocrine disrupting (ED) properties as a mode of action. Indeed, it acts as a selective estrogen receptor modulator (SERM) (Welshons et al. 2006); inducing changes in the expression level of sex steroid receptors, and modifying hormone synthesis and homeostasis (Rubin 2011). Estrogenic-like activities were involved in the recognized adverse effects of BPA including impairment of estrous cyclicity, learning and memory, metabolism and mammary gland function (ECHA 2017). More recently, the EFSA panel on food contact materials, after a systematic review of all BPA health

effects, followed by an assessment based on the biological relevance and weight of evidence (WoE) approaches, proposed to lower the temporary tolerable daily intake (TDI) dose from 4 µg/kg body weight (bw)/d (derived on the basis of renal effects in a developmental toxicity study) (EFSA 2015) to 0.2 ng/kg bw/d, based on effects on the immune system (EFSA 2023).

Restriction to the use of BPA prompted the design and production of alternative compounds, the majority of them being bisphenols with structural and functional similarities to BPA (analogues), although other compounds with different structures are also used. Regarding BPA replacement, different ways can be envisaged to provide alternatives to this substance. Those ways include the direct substitution of BPA by another substance, substitution by another plastic material or another polymer having similar properties to the starting polymer, substitution by another material, or another type of packaging or substitution by a process. In this review, only the first possibility is addressed. Although manufacturers have already started using chemicals alternative to BPA, in some cases after their inclusion in consumer products concerns are raised about their potential impact on human health and the environment. Some alternatives are being used industrially as monomers in the manufacture of epoxy resin and polycarbonate for many consumer products, such as food contact materials, personal care products, and thermal papers. Some BPA analogues have already been detected in the environment (Chen D et al. 2016; den Braver-Sewradj et al. 2020), with bisphenol S (BPS) and bisphenol F (BPF) being the most ubiquitous along with BPA (Russo et al. 2019; Catenza et al. 2021). Furthermore, several BPA alternatives have also been detected in human matrices worldwide, with various studies reporting human exposure in Europe (Karrer et al. 2020; Fillol et al. 2021; Govarts et al. 2023; Lobo Vicente et al. 2023). With increasing restrictions to the use of BPA, the tonnage and frequency of alternatives are expected to drastically increase in the coming years. However, in comparison to BPA, bisphenol alternatives are less well studied, and their potential toxicity remains unclear. The available toxicological information on a set of 24 BPA analogues of emerging interest was reported in a USA National Toxicology Program (NTP) Report and in a very comprehensive scoping review by Pelch K et al. (2019). The review analyzed the literature data till 2019, including data coming from literature searches conducted in the NTP Research Report 4 (NTP RR-04), and *in vitro* data produced by Tox 21 and Toxcast in the USA (Pelch KE et al. 2017). The authors noted that for substances such as bisphenol Z (BPZ), bisphenol E (BPE), bisphenol P (BPP), and bisphenol AP (BPAP), data on the potential toxicity are scarce, although some of them might be even more hazardous than BPA (Pelch K et al. 2019). In line with this suggestion, emerging data indicate that bisphenol B (BPB), BPF, and BPS may not be safer than BPA both in relation to ED (estrogenic, anti-estrogenic, androgenic, and anti-androgenic) activity and immunotoxic potential (Mustieles et al. 2020). Some BPA analogues were (or are being) evaluated by European regulators, including BPB (Serra et al. 2019; ECHA 2021), BPS (ECHA 2022), and BPF (ECHA 2022; Wiklund and Beronius 2022). In addition, BPA alternatives include also substances harboring flame-retardant properties, such as tetra-bromo bisphenol A (TBBPA) and tetra-chloro bisphenol A (TCBPA), which are derived from BPA by



halogenation. TBBPA was identified by ECHA as a carcinogen and has been placed in the candidate list of SVHC (ECHA 2023). These substances have been used to manufacture flame proofed epoxy resins, with TBBPA being a major flame retardant in terms of tonnage/use. It cannot be ruled out that other halogenated BPA analogues or alternatives (e.g. mono- to tetra-chloro BPA) can form in the environment due to the reaction of BPA with chlorinated waters (Plattard et al. 2021). In addition, photodegradation products of BPA chlorinated derivatives have been identified in wastewater treatment plant effluent under sunlight irradiation (Wan et al. 2020). Human exposure to chlorinated analogues was clearly demonstrated when they were detected in adipose tissue from women living in Southeast Spain (Fernandez et al. 2007) and later confirmed in placenta samples (Jiménez-Díaz et al. 2010). Subsequently, their presence in human tissue has been confirmed in several other countries (Andra et al. 2015). Therefore, the occurrence of mixed brominated/chlorinated bisphenols cannot be excluded. For most of these bisphenol alternatives, information on biotransformation and toxicokinetics (TK), ED activity, developmental neurotoxicity (DNT), genotoxicity, and carcinogenicity are either absent or too limited for a full hazard assessment and characterization.

A major goal of the Partnership for the Assessment of Risks from Chemicals (PARC, <https://www.eu-parc.eu>) is to advance the knowledge in the risk assessment of chemicals by closing data gaps on hazards for some substances and developing and integrating new approach methodologies (NAMs) (Marx-Stoelting et al. 2023). To this aim, a variety of studies are dedicated to the hazard assessment of substances included in a previously defined priority list (PARC D2.1, available at [https://www.eu-parc.eu/sites/default/files/2023-09/PARC\\_D2.1.pdf](https://www.eu-parc.eu/sites/default/files/2023-09/PARC_D2.1.pdf)) using several methodological approaches. On the one hand, for selected substances, e.g. BPA alternatives and some natural toxins, the identified regulatory data gaps comprising immunotoxicity and endocrine disruption, among other effects, will be filled by using test methods recommended by International Organizations (e.g. Organisation for Economic Cooperation and Development (OECD) Test Guidelines) in order to facilitate the regulatory acceptance of the data generated. On the other hand, NAMs will be developed to contribute to decrease uncertainties and improve those substances risk assessment. Indeed, seven distinct, but interacting scientific priorities areas were proposed to reach the goal of implementing NAMs into the new generation risk assessment (NGRA) (Escher et al. 2022). Those priorities are: production of TK data, with a focus on physiological based kinetic (PBK) modeling, development of additional adverse outcome pathways (AOPs) or AOP networks, development of advanced cell culture models including organ on chips, exposure, human susceptibility, data integration, new concepts in risk assessment (with cross-sectional activities, acceptance and assessment criteria, to update the guidance on how to integrate NAMs in human risk assessment), generally relevant for many chemicals, including BPA alternatives.

Among the group of BPA alternatives, some substances were prioritized based on the existent knowledge and discussions with the ECHA and EFSA experts, the relevant European stakeholders interested in the safety evaluation of these substances. The existence of evidence of human exposure and

the fact that their hazard had not been assessed by industry were considered as criteria for the selection. The specific project on the hazard assessment of BPA alternatives aims to fill the existing data gaps by providing a complete data set on four endpoints prioritized within PARC for human health, including ED activity, immunotoxicity, DNT, and genotoxicity/carcinogenicity. In addition, data on the toxicokinetic and metabolism will be produced for some compounds. A tiered approach will be followed for the hazard assessment of the selected BPA alternatives, with the collection of the published information representing the first step. In the scope of PARC, another project is addressing the effects of the same BPA alternatives on the ecosystem. With the objective of identifying research gaps on the potential deleterious effects of those compounds on the environment health, a review was recently published that describes the current knowledge and approaches used to assess their toxicity in invertebrate and vertebrate models (Adamovsky et al. 2024). Therefore, data and information from those alternative animal models are out of the scope of the present review.

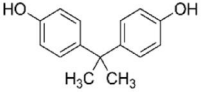
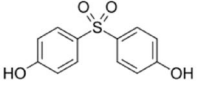
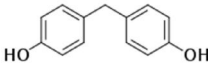
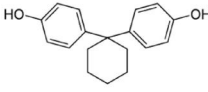
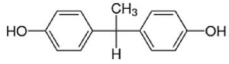
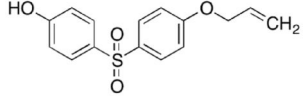
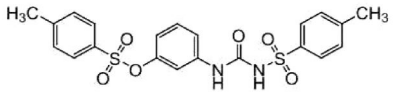
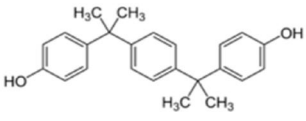
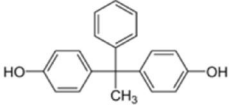
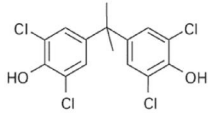
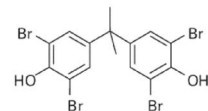
This review intends to critically address the current knowledge concerning the hazard of the BPA alternatives, particularly focusing on substances not currently subjected to any restriction and that were prioritized in PARC: BPE, BPAP, BPP, BPZ, bisphenol S 4-allyl ether (BPS-MAE), and TCBPA. Other alternative substances, such as Pergafast-201 and TBBPA, were included for specific endpoints (Table 1). Additionally, information related to BPA and its most commonly used analogues for which data are already available (e.g. BPS or BPF), is briefly reported for comparison. Besides offering a substantial update of the literature since the review by Pelch K et al. (2019), this review includes novel TK and metabolism information on the selected alternatives, drawing conclusions and identifying main gaps in knowledge about their hazard or risks. This information may guide the production of *in vitro* data that can be used as input for specific PBK modeling toward a NGRA approach, as foreseen by PARC, to avoid future regrettable substitutions.

## 2. ADME (absorption, distribution, metabolism, and excretion) processes of BPA alternatives

### 2.1. General considerations

The amount of any chemical that can reach and exert effects in target tissues leading to systemic toxicity is dependent on ADME processes. Already in 2010, the OECD adopted a guideline for the testing of chemicals' TK, which highlights the usefulness of TK data in many steps of the risk assessment of chemical substances (OECD 2010). Similar recommendations were reiterated over the years by the European Commission and other Agencies (European Commission 2012; EFSA 2014), placing TK among the key research priority topics, to accelerate the move toward the reduction of animal testing (3R concept) and promote a more mechanistic understanding of toxicology. More recently, EFSA pointed out that the production of TK data, with a focus on PBK modeling, is one among the seven distinct, but interacting scientific priorities areas proposed for reaching the goal of implementing NAMs into the NGRA (Escher et al. 2022).

**Table 1.** List of prioritized BPA alternatives including: CAS number, common and IUPAC name, chemical structure and type of central carbon (quaternary/not quaternary).

CAS Number	Common Name IUPAC Name	Chemical structure and central carbon: quaternary/not quaternary	
80-05-7	*BPA; Bisphenol A/ 4-[2-(4-hydroxyphenyl)propan-2-yl]phenol		●
80-09-1	*BPS; Bisphenol S/ 4,4'-sulphonyldiphenol		
620-92-8	*BPF; Bisphenol F/ 4,4'-methylenediphenol		●
843-55-0	BPZ; Bisphenol Z/ 4-[1-(4-hydroxyphenyl)cyclohexyl]phenol		●
2081-08-5	BPE; Bisphenol E/ 4-[1-(4-hydroxyphenyl)ethyl]phenol		●
97042-18-7	BPS-MAE/ 4-(4-prop-2-enoxyphenyl)sulfonylphenol		
232938-43-1	Pergafast 201/ [3-[(4-methylphenyl) sulfonylcarbamoylamino]phenyl] 4-methylbenzenesulfonate		
2167-51-3	BPP; Bisphenol P/ 4-[1-(4-hydroxyphenyl)-1-phenylethyl]phenol		●
1571-75-1	BPAP; Bisphenol AP/ 4-[1-(4-hydroxyphenyl)-1-phenylethyl]phenol		●
79-95-8	TCBPA; tetrachlorobisphenol A/ 2,6-dichloro-4-[2-(3,5-dichloro-4-hydroxyphenyl)propan-2-yl]phenol		●
79-94-7	TBBPA; tetrabromobisphenol A/ 2,6-dibromo-4-[2-(3,5-dibromo-4-hydroxyphenyl)propan-2-yl]phenol		●

\*BPA and its analogues BPS and BPF, all data reach substances, are used in this review for comparison (substances in grey). Further details on the prioritization process can be found at: [https://www.eu-parc.eu/sites/default/files/2023-08/PARC\\_AD5.1.pdf](https://www.eu-parc.eu/sites/default/files/2023-08/PARC_AD5.1.pdf).

Over the last decades, the increasing knowledge about the ADME characteristics of BPA has been extremely useful to decrease uncertainties associated with the derivation of health-based values. Indeed, TK knowledge allowed that species and age-related differences in BPA systemic bioavailability can be considered, and these studies also contributed to the development of adequate PBK modeling to derive a human equivalent dose (HED) (EFSA 2010, 2015, 2023). The considerable data available as regards BPA TK has been recently used as a case study to show

how kinetics data, integrated with a computational workflow, can facilitate quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) (Loizou et al. 2021).

## 2.2. Information available on BPA-alternatives kinetics

As regards BPA alternatives, data are available only for a limited number of substances. When useful, these have been

briefly cited here for comparison. The best documented compound is BPS, which, for this reason, has not been included in the priority list of substances considered within the PARC project. A review has recently been published (Beausoleil et al. 2022) indicating that the estimated BPS human plasma clearance (0.92 L/min) is lower than that of BPA, suggesting a less efficient elimination (Grandin F et al. 2017; Gayrard et al. 2020) and potentially increased toxicity (e.g. on reproductive system) compared to BPA (Beausoleil et al. 2022). Studies comparing BPA and BPS ADME data following oral or dermal exposure, have clearly shown that, despite the similarities between the two molecules, significant TK differences exist (Thayer et al. 2015; Karrer et al. 2018; Oh et al. 2018; Waidyanatha et al. 2018; Liu J and Martin 2019; Champmartin et al. 2020; EFSA 2020; Khmiri et al. 2020; Reale et al. 2020). These observations underline the need to acquire ADME data for all BPA alternatives. In addition, species differences in TK have been reported (Sonker et al. 2021), which should be considered in the risk assessment process, when rodent data are used, as already achieved for BPA with the derivation of the HED. Three TK models have been proposed for BPS (Karrer et al. 2018; Oh et al. 2018; Gingrich et al. 2021). The model of Oh et al. (2018) has the advantage of being based on human data, but it is not a physiologically based model. The two other models are PBK but are based on quantitative structure–activity relationship (QSAR) and/or animal data (Corbel et al. 2013; Grandin FC et al. 2018; Gingrich et al. 2021). More recently, a model of human dermal absorption of BPA, BPS, BPF, and bisphenol AF (BPAF) was published (Hu M et al. 2023), in which a parallel-layered skin compartment model was integrated into the existing PBK models for BPS (Karrer et al. 2018). It can be derived that, after dermal exposure, women experienced an internal exposure to BPS characterized by an area under the curve (AUC, i.e. the definite integral of the concentration of a chemical in blood plasma as a function of time) and  $C_{\max}$  (the highest concentration in blood) values 11-fold lower when compared to BPA. For BPF and BPAF, the same parameters were 3- and 40-fold lower than for BPA, respectively (Hu M et al. 2023).

Another recent study investigated the TK behaviors of mixtures of 11 BPA alternatives (among which BPS, BPAF, BPB, BPF, BPZ, BPAP, BPP, BPM, and BPFL) following intravenous or oral administration to piglets (Gély et al. 2023). The systemic concentration reached by each substance was compared with that of BPA using the TK predicted by the generic High Throughput Toxicokinetics (HTTK) model package. The findings indicated that, at the same oral dose, all the tested BPA analogues produced a higher systemic exposure (measured as AUC) than BPA (2- to 4-fold for BPAF, BPB, and BPZ; 7- to 20-fold for BPAP, BPP, and BPF and 150-fold for BPS). The observed differences were attributed to variations in the systemic bioavailability of the substances. The results also indicated that conversely to other bisphenols, BPP, bisphenol M (BPM), and bisphenol FL (BPFL) showed a low urinary excretion, an important aspect to consider in predicting human exposure based on urine biomonitoring (Gély et al. 2023).

### 2.3. Placental and blood–brain-barrier (BBB) transfer

Some publications have documented the transfer of BPA and its alternatives through the placental barrier and the BBB. Using an *ex vivo* human placental perfusion model (Gély et al. 2021), bisphenols were shown to greatly differ in placental transport efficiency, with BPAP, BPE, BPF, BPB, and BPA being transferred by passive diffusion. However, BPZ, BPP, and BPAF, among others, had a lower transfer, suggesting weak diffusional permeability and/or the action of efflux protein. The placental transfer rate of BPS was also very limited (Gély et al. 2021): only 0.40% of the maternal BPS dose was transferred, 10 times lower than that of BPA (Grandin FC et al. 2019).

Differences in TK among 15 bisphenols (BPA, BPF, BPAP, BPP, BPZ, BPE, BPS, BPAF, and among others) have also been demonstrated in humans by studying 60 triplets of maternal plasma, cord plasma, and placenta samples from pregnant women in South China (Pan et al. 2020). Besides BPA, also BPS, BPAF and BPE were measured in all maternal plasma samples and detected with high frequency (from 80 to 100%) in mother–child pairs. Significant correlations between maternal plasma and cord plasma concentrations were observed for the most frequently detected bisphenols. The comparison between maternal and cord blood levels of bisphenols showed a different behavior regarding placental transfer efficiencies, with BPAF showing the highest efficiency and suggesting potential accumulation in the fetus (Pan et al. 2020).

Information about the transport across the BBB is extremely limited and no published data have been found for the BPA alternatives targeted in this review. However, docking modeling suggests that, based on their general structure, bisphenols are expected to bind to the same cavity of the breast cancer resistance protein (BCRP) efflux transporter, similarly to BPA and other inhibitors, in human *in vitro* BBB models (Nickel and Mahringer 2014; Engdahl et al. 2021).

### 2.4. Conjugation pathways: a major metabolic reaction for BPA alternatives

Based on the large database on BPA TK, it has been assumed that structurally related alternatives readily undergo conjugation to corresponding glucuronides and/or sulfates. Indeed, given the phenolic nature of these substances, no first-step oxidation is required prior to conjugation. The occurrence of conjugation pathways was demonstrated for BPA and several bisphenols (Knaak and Sullivan 1966). BPA mono-glucuronide (BPA-G) is the major metabolite of BPA in rodents (Snyder et al. 2000; Zalko et al. 2003) as well as in humans and other primates (Völkel et al. 2002; VandeVoort et al. 2016). This pathway was also extensively documented for BPS *in vitro* and *in vivo*, including in humans (Gys et al. 2018; Waidyanatha et al. 2018), as well as for some other alternatives, including BPF (Cabaton et al. 2006, 2008), BPAF (Li M et al. 2013), and TBBPA (Fini et al. 2012). In rodents, BPF was found to be efficiently metabolized into a sulfate conjugate following oral administration (Cabaton et al. 2006), but for most analogues, the relative contribution of glucuronidation vs. sulfation was not fully characterized. Notably, the



occurrence of doubly conjugated metabolites (glucuronide and/or sulfate) was also demonstrated. Conversely to the parent BPA molecule, BPA-G was shown not to be an activator of nuclear ERs (Matthews et al. 2001). However, some reports have suggested that BPA-G has biological effects *in vitro* (Viñas et al. 2013; Boucher et al. 2015), but these should be considered with much caution, due to the high BPA-G concentrations tested and the possible back conversion of BPA-G into its unconjugated form that has been demonstrated *in vivo* (Corbel et al. 2015; Gauderat et al. 2016). Consequently, BPA glucuronidation is generally regarded as a detoxification pathway and, likewise, the sulfation pathway, despite the lack of conclusive published data. Of note, some conflicting results exist about the ER activities of BPS-sulfate (Kang et al. 2014; Skledar et al. 2016). With the exception of the data reported for few BPA alternatives, very limited information exists on the metabolic pathways followed by the substances selected to be studied under the PARC project.

### 2.5. Oxidative metabolism of BPA alternatives

The occurrence of oxidations, driven by cytochrome P450 (CYP) activity, has also been clearly demonstrated for BPA, as well as for few other alternatives. These pathways are still very much unexplored and warrant further investigations based on chemical similarities with BPA. Indeed, bisphenols oxidation may trigger the production of reactive intermediates and metabolites, which may explain some observed reactive oxygen species (ROS) related effects, such as immunotoxic and genotoxic effects (Zalko et al. 2006). Several (and distinct) oxidative metabolic pathways have been identified based on BPA studies. Their occurrence is closely conditioned by the chemistry of the molecules, which brings useful clues for further investigations to be carried out on not yet studied alternatives.

First, oxidation can occur ortho to one of the hydroxy functions, leading to the formation of a reactive catechol, and eventually of the corresponding quinone (Jaeg et al. 2004; Schmidt et al. 2013). Since these two metabolites may form DNA adducts (Atkinson and Roy 1995), this specific phase I reaction can be considered as a bioactivation route, unless BPA-catechol is detoxified by catechol-O-methyltransferase (COMT) activities (Zalko et al. 2003). Although catechols and related chemically reactive intermediates are quantitatively minor metabolites, few studies have demonstrated the occurrence of this pathway for BPS, BPF, BPAF, BPZ, BPB, and BPC (Yoshihara et al. 2004; Schmidt et al. 2013). Of note, BPA analogues bearing bulky substituents ortho to the hydroxy functions, as it is the case of tetra-halogenated BPA (TBBPA and TCBPA) and tetra-methyl BPA (TMBPA), are not expected to be prone to follow this ortho-oxidation pathway.

A second possibility is an oxidation taking place opposite to the hydroxy function, and involving a P450 mediated ipso-addition, immediately followed by an ipso-substitution rearrangement. For chemical reasons, this pathway can occur only for bisphenols that possess a central quaternary carbon.

It was clearly demonstrated for BPA (Jaeg et al. 2004), with its molecular mechanisms fully detailed by Nakamura et al. (2011). Ultimately, this peculiar pathway leads to a cleavage of the BPA molecule into two smaller metabolites, namely isopropenyl-phenol and hydroxycumyl alcohol, the latter having also been suggested to be an active metabolite at the level of endoplasmic reticulum (Nakamura et al. 2011). This first-step oxidation position is also concomitant with the production of glutathione conjugates hinting for the formation of reactive intermediates (Jaeg et al. 2004), as well as the formation of dimeric structures (Jaeg et al. 2004; Yoshihara et al. 2004). This metabolic pathway was also demonstrated to occur for TBBPA (Zalko et al. 2006). Notably, based on TBBPA studies, it is likely that this pathway is enhanced by the presence of halogens (Zalko et al. 2006). Data still lack about many BPA alternatives for which there are fair chances this pathway occurs, i.e. all molecules which possess a central quaternary carbon (e.g. BPPB, BPAP, and bisphenol BH (BPPH)), and particularly mono- to tetra-halogenated BPA, including TCBPA. The further glucuronidation or sulfation of these hydroxylated metabolites was also reported *in vitro* for BPA as well as other analogues such as BPS and BPF (Cabaton et al. 2008; Skledar et al. 2016; Gys et al. 2018).

Finally, it should be mentioned that bisphenols oxidation can also occur at the level of a methyl group next to the central carbon, as first demonstrated for BPA (Zalko et al. 2003). This may occur for any analogue in which the central carbon bears one or two methyl substituents (e.g. BPE, BPB, BPM, BPAP, and halogenated BPA analogues). Although data lack for alternatives other than BPA, it is unlikely that this specific oxidation position would result in a bioactivation process, conversely to the previously detailed pathways.

### 2.6. Isoform specific metabolism of BPA analogues

Besides the above-described major knowledge gaps that still exist for several BPA alternatives, additional information about isoform-specific metabolism (i.e. identification of the human isozymes involved in BPA alternatives metabolism) with the related biochemical parameters (including the intrinsic clearance) is also missing for most substances. This kind of data can be obtained by using human-derived *in vitro* models (e.g. recombinant enzymes, human microsomes, and other sub-cellular fractions from various organs) and gives a fundamental input for PBK modeling to estimate internal dose and identify human variability (Testai et al. 2021). As regards phase II metabolism, available data suggest that BPA analogues are detoxified by different UDP glucuronosyltransferase (UGT) isoforms with respect to BPA, that is UGT2B15, followed by UGT1A9, UGT2B7, UGT1A1, and UGT2B4 (Hanioka et al. 2008). The glucuronidation of BPAF, at 10  $\mu$ M, was assessed using 12 human recombinant UGTs. UGT2B7 was found to be the most active, while no BPAF glucuronide was formed when using UGTs 1A4, 1A6, 1A7, and 1A10 (Li M et al. 2013). More recently testing of a range of BPAF concentrations (0.1–50  $\mu$ M), a1A3, 2B7, 2B17, 1A10, and 2A1 showed similar intrinsic clearance value (calculated as the  $V_{max}/K_m$

ratio), although the ones with the highest affinity, i.e. 2B7 and 2B17 were found to be the most relevant at low exposure concentration (Skledar et al. 2019). Of note, UGT2B17, besides being a hepatic isoform, also accounts for >50% of the total intestinal UGTs (Kasteel et al. 2020), indicating that the pre-systemic detoxification, limiting the bioavailability of the toxic parent, can be extremely relevant. Other isoforms were mainly responsible for BPS (UGT1A9) and BPF glucuronidation (UGT1A10) (Skledar et al. 2015). No data are available on the isoform specific glucuronidation of the BPA alternatives selected in the PARC project. However, since the available data seem to indicate differences among similar molecules, it is worthwhile to investigate it, considering the interindividual differences as well as differences in the life stages, since some UGT isoforms are not fully expressed in the neonatal period.

The metabolism of BPS, BPF, and BPAF was also investigated using human liver microsomes (HLM) and human intestine microsomes (HIM), showing that the intestine contributes less than the liver to their overall metabolism (Skledar et al. 2016). It was also demonstrated that the relative efficiency of detoxification was highly dependent upon the exposure concentration: at lower substrate concentrations, representative of human exposure, the glucuronidation of BPAF was the most effective while that of BPS was the least effective among all the tested analogues (Karrer et al. 2018).

Sulfation of BPA is catalyzed predominantly by the sulfotransferase (SULT) SULT1A1, SULT2A1, and SULT1E1 (Suiko et al. 2000; Nishiyama et al. 2002). For BPA analogues, no data on single recombinant SULTs are available for any bisphenol to explore the isoform specificity of this reaction. However, sulfation was investigated for BPA, BPS, and BPF using human liver cytosol (HLC). By comparing sulfation with previously reported kinetic parameters for glucuronidation, it was shown that BPA and BPF sulfation was less efficient than glucuronidation, while for BPS the intrinsic clearance was closer between the two reactions (Durcik, Skledar, et al. 2022).

For phase I oxidative pathways, only limited data are available, and studies have focused only on the CYP-mediated formation of hydroxylated metabolites. In addition to the study of Nakamura et al. (2011), highlighting the role of the CYP3A and CYP2D6 isoforms, Schmidt et al. (2013) compared the formation of hydroxylated metabolites for BPA and BPF, BPAF, and BPZ using single CYPs isoforms (CYP3A4, CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP2E1) and a single substrate concentration. All CYPs tested were active, suggesting that hydroxylation reactions are not specifically catalyzed by one specific CYP and ipso-substitution reactions of bisphenols were not found to be major pathways in this study. However, the production of reactive intermediates through this specific pathway may lead to their immediate binding to endogenous macromolecules and/or to the cleavage of the parent molecule into smaller metabolites, therefore, complicating the qualitative and quantitative monitoring of the contribution of the ipso-substitution pathway in bisphenols metabolism. Despite bisphenols structural proximity, differences were found to exist: CYP1A2 and CYP2C9 mainly catalyze

BPA and BPF hydroxylation, whereas BPAF is mainly hydroxylated by CYP3A4 and to a lower extent by CYP2C9, BPZ is mainly metabolized by CYP2C8 (Schmidt et al. 2013); BPS has been reported to be hydroxylated by CYP3A4 and CYP2C9 (Skledar et al. 2016). However, neither kinetic parameters nor their dependence on the concentration of the different bisphenols were derived.

## 2.7. Halogenated bisphenols and other specific alternatives

Halogenated bisphenols are a key sub-family of analogues, which mainly comprises TBBPA, a substance that still accounts for 50% of the flame retardants market, but also chlorinated and mixed Br/Cl bisphenols. The kinetics of TBBPA in humans have been reported following a single oral dose of 0.1 mg/kg bw TBBPA (five individuals) and in rats administered a single oral dose of 300 mg/kg bw TBBPA (Schauer et al. 2006). TBBPA was not present at detectable concentrations in any of the human plasma samples, while TBBPA-glucuronide and TBBPA-sulfate were identified as metabolites of TBBPA in blood and urine of both humans and rats, suggesting a low systemic bioavailability of the parent compound. In humans, a higher frequency of detection and levels were reported for the TBBPA-glucuronide. The absorption, as well as the conjugation, were rapid processes ( $C_{max}$  for the glucuronide was <4 h in humans and <3 h in rats), whereas the urinary excretion was slow. In rats, in addition to TBBPA-glucuronide and sulfate, a diglucuronide of TBBPA, a mixed glucuronide-sulfate conjugate of TBBPA, tri-bromo bisphenol A, and the glucuronide of tri-bromo bisphenol A were also present in low concentrations. TBBPA in rat plasma showed a half-life of 13 h (Schauer et al. 2006). Low concentrations of TBBPA have been shown to inhibit BCRP-mediated transport in rat brain capillaries (Cannon et al. 2019). This information is relevant since blocking BCRP-mediated efflux is likely to result in higher chemical exposure of the brain.

Halogenation likely enhances the potential of such bisphenols to undergo metabolic activation through phase I reactions, with ROS production and possible links with induced inflammation, immunotoxicity, and genotoxicity. In addition, TCBPA, TBBPA as well as TBBPA mono-sulfate were demonstrated to be potent activators of the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) (Riu, Le Maire, et al. 2011) unlike most other BPA analogues, which primarily target an ER response. Thus, halogenated BPA may trigger a modulation of energy metabolism through a distinct mode of action.

Alternatives used in thermal papers (structurally close to bisphenols: BPS-MAE and BPS-isopropyl (BPS-IP), or less close: Pergafast-201) form another sub-family of concern. Little is known about their fate, especially as regards the Pergafast family. Note that compounds like Pergafast-201 are also expected to break down into two daughter molecules in solution, therefore, requiring the study of by-products in addition to the mother molecule (Eckardt et al. 2020).

**Table 2.** Synthesis of the range or single IC<sub>50</sub>/AC<sub>50</sub> values reported in the literature for each BPA alternative, grouped by *in vitro* assay categories.

	BPAP	BPE	BPP	BPS-MAE	BPZ	TBBPA	TCBPA
ERα binding affinity	255 nM to 2.6 μM	5.3 μM	152 nM to 0.671 μM	Inactive	11–80 nM	250 nM	2.8 μM
ERα reporter gene assay agonism	351 nM to 2 μM	0.102–7.43 μM	0.64 μM (REC <sub>20</sub> )	Inactive	3.7 pM to 8.63 μM	Inactive/19 μM	20 nM to 47.60 μM
Antagonism	24.54 nM (IC <sub>20</sub> )		3.8 nM (RIC <sub>20</sub> ) to 1.91 μM			0.6 μM	
E-screen (proliferation)	0.39 μM	0.1–10 μM	NA	NA	0.11 μM	124 μM	45.8 μM
ERα gene expression	NA	NA	NA	NA	NA	↑ 1 pM	↑ 1–10 μM/↓ 25 μM
ERα protein expression	NA	NA	NA	NA	NA	Inactive	↑ 10 nM to 1 μM
ERβ binding affinity	62.1–68 nM	3.9 μM	105 nM	Inactive	128 nM to 1.1 μM	NA	NA
ERβ reporter gene assay agonism	0.17–0.61 μM	94 nM to 4.6 μM		Inactive	18 nM to 0.5 μM	Inactive	1.15–68 μM
Antagonism	595 nM		0.93–1.81 μM		1.16 μM		
ERβ gene expression	NA	NA	NA	NA	NA	↑ 100 pM to 10 μM	NA
ERRγ binding affinity	120 nM	4.78 nM	320 nM	NA	13.4 nM	NA	NA
ERRγ reporter gene assay	Inactive	0.69 μM	Inactive	NA	5.24 μM	Inactive	Inactive
ERRγ gene expression	NA	NA	NA	NA	NA	↑ 100 pM	NA
GPER proliferation assay	NA	NA	NA	NA	NA	↑ 10–50 nM	Inactive
GPER gene expression	NA	NA	NA	NA	NA	↑ 1 pM to 10 μM	↑ 10 nM to 10 μM
GPER protein expression	NA	NA	NA	NA	NA	↑ 10 nM	↓ 1 nM/↑ 10 nM to 1 μM
AR binding affinity	5.4–>10 μM	>10 μM	409 nM	Inactive	1.6–6.68	1–10 μM	NA
AR reporter gene assay antagonism	8.5–40 μM	0.99–10 μM	2.0–10.8 μM	Inactive	3.58 nM to 7.3 μM	0.4–0.982 μM	100 nM (RIC <sub>20</sub> ) to 13.4 μM
AR anti-proliferative assay	NA		NA	NA	NA	Inactive	Inactive
AR protein expression	↓ 16.0 μM	NA	NA	NA	NA	NA	NA
PR binding affinity	330 nM	inactive	896 nM	NA	1.89 μM	NA	NA
PR reporter gene assay antagonism	23.1 μM	inactive	7.18 μM	NA	7.64 μM	78 nM (RIC <sub>20</sub> )	27 nM (RIC <sub>20</sub> )
PR gene expression	↑ 1 μM	↑ 1 μM	↑ 1 μM	NA	↑ 1 μM	NA	↑ 1–25 μM/↓ 10 nM to 10 μM
PR protein expression	NA	NA	NA	NA	NA	↑ 30 μM	↑ 10 μM
GR binding affinity	30.1 nM	>10 μM	213 nM	NA	226 nM	0.5 μM	NA
GR reporter gene assay agonism	158 nM	Inactive/↑	0.32 nM (LOEC)	NA	1.99 μM (LOEC)	Inactive	Inactive
Antagonism	8.1 μM		17.8 nM (RIC <sub>20</sub> ) to 16.1 μM		8.8–22 μM	22 nM	
GR gene expression	NA	NA	NA	NA	NA	↑ 1–100 pM	NA
PPARγ binding affinity	>10 μM	Inactive	Inactive	NA	Inactive	0.7–5.7 μM	6 μM
PPARγ reporter gene assay agonism	NA	NA	NA	NA	NA	0.2–397 μM	0.3–1.28 μM
PPARγ gene expression	NA	NA	NA	NA	NA	↑ 1–20 μM	NA
PPARγ protein expression	NA	NA	NA	NA	NA	↓ 10 μM/↑ 10 nM to 30 μM	↓ 1 nM
PPARα/β/δ binding affinity	NA	NA	NA	NA	NA	68 μM	102 μM
PPARα/β/δ reporter gene assay	NA	NA	NA	NA	NA	25–50 μM	50 μM
PPARα gene expression	NA	NA	NA	NA	NA	↓ 1 μM	NA
PPARβ/δ gene expression	NA	NA	NA	NA	NA	↑ 1 μM	NA
PXR binding affinity	1.06 μM	>10 μM	2.45 μM	NA	2.15 μM	NA	NA
PXR reporter gene assay agonism	9.7 (REC <sub>20</sub> ) to 65.9 μM	Inactive	6.3 (REC <sub>20</sub> ) to 19 μM	NA	8.7–10.2 μM	11.97–123.1 μM	8.49–24.1 μM

AR: androgen receptor; ER: estrogen receptor; ERR: estrogen-related receptor; GPER: membrane G protein-coupled ER; GR: glucocorticoid receptor; LOEC: lowest observed effective concentration; NA: not assessed; PXR: pregnane X receptor; PPAR: peroxisome proliferator activated receptors; PR: progesterone receptor; REC: relative effective concentration; RIC: relative inhibitory concentration. In case of IC<sub>20</sub>/AC<sub>20</sub> values, they are specified; in addition, values related to genes/protein expression are concentrations at which significant effects were observed. The detailed list of all the assays and the related publications is shown in Table A1.

## 2.8. Concluding considerations on the kinetics of BPA alternatives

Apart from the bioactivation issues that remain unexplored for many bisphenol alternatives, a better knowledge of the metabolic pathways of these substances may help in (i) decreasing the uncertainties in risk assessment of BPA alternatives, accounting for possible species differences between human and data obtained with animal models, following the example of BPA; (ii) providing key information to modelers, about the processes likely to impact actual internal biologically active doses; and (iii) identifying new candidate biomarker metabolites that have the potential to be used as exposure biomarkers to enhance human biomonitoring strategies. Of note, exposure to bisphenol alternatives may also result in a modulation of liver metabolic capacities, by inducing or repressing phase I and II xenobiotic metabolizing enzymes (XME) expression and functionality. This may well be a mode of action for these candidate metabolic disruptors, since these enzymes are also deeply involved in endogenous metabolisms, including that of many hormones. In that sense, bisphenols and their metabolites may act as metabolism disrupting chemicals (MDC) not only through the activation of specific receptors (ER, PPAR), but also through modulating liver XME activities.

*In vitro* systems are increasingly used to characterize the toxicological profile of chemicals, including bisphenol alternatives, and to collect information on their mechanism of action. However, frequently the metabolic competence and/or the activities of transporters in these systems are unknown. *In vitro* assays are generally carried out without considering the *in vitro* kinetics of the tested substance, which would enable the measurement of the actual intracellular concentration of the active/toxic compound(s). Consequently, the nominal concentration is typically used for *in vitro* dose–response curves (Kramer et al. 2015). How the use of the nominal concentrations could lead to the misinterpretation of results has been reported in a number of publications (Wilmes et al. 2013; Kramer et al. 2015; Pomponio et al. 2015; Truisi et al. 2015; Gouliarmou et al. 2018); in addition, the OECD, among others, has clearly pointed out that the metabolic characterization of *in vitro* systems and biokinetics measurements are pivotal requirements, in its Guidance Document on Good *In Vitro* Method Practices (GIVIMP) (OECD 2018). The importance of biotransformation processes in determining the bioavailable concentration of parent bisphenols is highlighted by the fact that only the parent substances (in most cases), but not their conjugates, are able to activate nuclear receptors (NRs). These considerations as well as the potential formation of reactive metabolites, should be considered any time an *in vitro* system is used when interpreting data and trying to extrapolate to the actual *in vivo* situation. Therefore, the observed *in vitro* effects should be interpreted with caution, if the intracellular context of exposure (actual concentration, presence of metabolites) was not assessed, especially in the absence of effects.

## 3. Endocrine disrupting activity of BPA alternatives

### 3.1. General considerations

Based on the WHO definition of EDCs (WHO 2013), the European Commission established three criteria that a substance should fulfill for ED classification. These criteria are related to endocrine activity, adverse effect and the biological plausible link between the endocrine activity and the adverse effect (ECHA and EFSA 2018). BPA has been classified as SVHC for its ED activity. Despite data have been already produced and analyzed for ED-related adverse effects of several BPA alternatives (Pelch KE et al. 2017; Pelch K et al. 2019), the following section aims to update and describe the currently available evidence on BPA alternatives' ED activity on NRs, thyroid hormones (THs) homeostasis and steroidogenesis. Only publications using chemical standards of compounds identified as priority in PARC (i.e. BPE, BPP, BPZ, BPAP, BPS-MAE, and TCBPA) have been considered, excluding assessment of ED activity of matrix extracts of any origin (thermal paper, sludge, etc.). Studies describing effects of TBBPA were also considered for comparison due to chemical similarity with TCBPA.

### 3.2. Interaction and effects on nuclear steroid, PPARs, and other receptors

The following sections describe effects exerted by BPA alternatives on these NRs based on *in vitro* studies. Data are summarized in Table 2 and reported in detail in Table A1.

#### 3.2.1. Estrogen receptors (ER $\alpha$ , ER $\beta$ , ERR $\gamma$ , GPER)

The estrogenic activity of BPA is certainly the best characterized; therefore, effects of BPA alternatives on nuclear estrogen receptors (ER $\alpha$ , ER $\beta$ ) are the most investigated. The relative binding affinities of TCBPA and TBBPA, assessed in competitive binding experiments on ER $\alpha$  isolated from breast MCF-7 cells, were much lower than that of BPA (Samuelson et al. 2001; Olsen et al. 2003). By using ER $\alpha$  affinity columns, only TCBPA bound to the receptor (Riu, Le Maire, et al. 2011).

In a gene reporter assay in MCF-7 cells, TCBPA displayed stronger estrogenic activity than BPA while TBBPA had weak activity. In contrast, TBBPA 10  $\mu$ M exhibited anti-estrogenic activity in presence of E2 10 pM (Kitamura et al. 2005) and in a yeast two-hybrid assay (van Leeuwen et al. 2019). However, TBBPA did not display any agonist/antagonist activity in several other studies (Hamers et al. 2006; Li J et al. 2010; Lee HK et al. 2012; Molina-Molina et al. 2013; Ruan et al. 2015; Grimaldi et al. 2019; Durcik, Hiti, et al. 2022). TCBPA had no activity in a two-hybrid yeast assay (Li J et al. 2010), whereas it was confirmed to be an ER $\alpha$  agonist using stably transfected cell lines (Riu, Grimaldi, et al. 2011; Riu, Le Maire, et al. 2011; Molina-Molina et al. 2013; Pelch KE et al. 2019; Durcik, Hiti, et al. 2022), and in a bioluminescence yeast estrogen screen assay (Ruan et al. 2015) showing a potency generally lower than that of BPA. TCBPA had agonist potency lower than BPA also for ER $\beta$  in transfected HGELN-ER $\beta$  cells (Riu, Le Maire, et al. 2011) while TBBPA was inactive (Pelch KE et al.



2019) or had very limited effects on both ERs (Riu, Grimaldi, et al. 2011; Riu, Le Maire, et al. 2011).

BPZ displayed a dose-dependent binding to ER $\alpha$  comparable to BPA (Böckers et al. 2020). BPAP and BPZ, showed to be highly selective, with BPAP having a higher affinity for ER $\beta$  and BPZ for ER $\alpha$  (Liu X et al. 2019, 2021). Another report confirmed the higher affinity of BPZ for ER $\alpha$  while no binding to ER $\beta$  was observed for BPAP (Keminer et al. 2020). BPZ had a stronger binding affinity than BPP for ER $\alpha$  in MVLN cells, whereas BPAP was inactive (Lin et al. 2021). BPE was a weak binder of ER $\alpha$  in a yeast two-hybrid assay, while displaying a strong binding activity in a cell-free system (Hashimoto et al. 2001). In addition, BPE and BPP had high binding affinity for both ERs, with BPE being more potent; interestingly, BPE was also a strong binder for ERR $\gamma$ , with a potency similar to that of BPA. The affinity of the other alternatives for ERR $\gamma$  was lower, with the following decreasing order: BPZ > BPAP > BPP (Liu X et al. 2019).

Agonistic effects of BPE for ER $\alpha$  were observed in several *in vitro* models, with a potency often comparable or higher than BPA (Rosenmai et al. 2014; Ruan et al. 2015; Conroy-Ben et al. 2018; van Leeuwen et al. 2019; Chen Q et al. 2020; Durcik, Hiti, et al. 2022). Similar results were observed also in transfected cells for both ERs (Grimaldi et al. 2019; Kojima et al. 2019; Pelch KE et al. 2019).

BPZ had similar or higher agonist potency as BPA for ER $\alpha$  in transfected MCF-7 cells and yeast assays (Mesnage et al. 2017; Conroy-Ben et al. 2018; Pelch KE et al. 2019; van Leeuwen et al. 2019). Conversely, BPZ was less potent than BPA for ER $\beta$  in transfected HepG2 cells (Pelch KE et al. 2019), whereas in other transfected cells BPZ was a strong agonist for both ERs (Grimaldi et al. 2019; Kojima et al. 2019). The transactivation potency for ER $\alpha$  was confirmed in other reporter gene assays but BPZ was almost inactive for ER $\beta$  (Chen Q et al. 2020; Keminer et al. 2020; Liu X et al. 2021) or, rather with antagonist activity (Liu X et al. 2021). Opposite evidence was observed in human embryonic kidney HEK293 cells over-expressing ER $\alpha$ , where BPZ (25  $\mu$ M) reduced ER $\alpha$  activity with time (Böckers et al. 2020).

In various reporter gene assays, BPAP had a lower agonist potency than BPA for ER $\alpha$  (Mesnage et al. 2017; Grimaldi et al. 2019; Kojima et al. 2019; Pelch KE et al. 2019; Liu X et al. 2021), whereas it was completely inactive in a yeast two-hybrid assay (van Leeuwen et al. 2019). However, BPAP displayed antagonist activity for ER $\alpha$  in MVLN cells (Chen Q et al. 2020), as well as agonist (Grimaldi et al. 2019; Kojima et al. 2019) or antagonist activity (Liu X et al. 2021) for ER $\beta$  using different transfected cells.

BPP was inactive for ER $\alpha$  in two different *in vitro* systems (Pelch KE et al. 2019; van Leeuwen et al. 2019) whereas it exhibited antagonist activity for ER $\alpha$  in reporter gene assays (Grimaldi et al. 2019; Chen Q et al. 2020). BPP was also antagonist of ER $\beta$  in transfected cells (Grimaldi et al. 2019; Pelch KE et al. 2019). BPP agonism for ER $\alpha$  was observed only up to 1  $\mu$ M, then decreasing its activity with the dose, but it was a strong antagonist for both ERs (Kojima et al. 2019).

BPS-MAE has been limitedly investigated, with data reporting its inactivity as binder or as agonist/antagonist on both receptors (Pelch KE et al. 2019; Keminer et al. 2020).

BPA has a high affinity for ERR $\gamma$  (Takayanagi et al. 2006). In transfected HELN-ERR $\gamma$  cells, BPE had the highest potency, followed by BPZ, whereas BPAP, BPP, TCBPA, and TBBPA were inactive (Grimaldi et al. 2019), confirming for the latter two compounds what was previously observed in a yeast assay (Li J et al. 2010).

By the E-screen assay, assessing the proliferation induction of MCF-7 ER $\alpha$  positive cells, TCBPA displayed a very low proliferative potential, only at the highest concentration tested, while TBBPA had no effects (Samuelsen et al. 2001; Olsen et al. 2003; Molina-Molina et al. 2013). However, in another report, TBBPA exerted both estrogenic and anti-estrogenic potential (in presence of E2) on MCF-7 cell growth (Krivoshiev et al. 2016). In contrast, BPE, BPZ, and BPAP strongly promoted cell growth (Hashimoto et al. 2001; Mesnage et al. 2017). To our knowledge, no evidence is available on the proliferative potential of BPP and BPS-MAE. Neither TCBPA (10  $\mu$ M) nor TBBPA (30  $\mu$ M) affected ER $\alpha$  protein expression in MCF-7 cells (Olsen et al. 2003). However, in same cells, TCBPA induced ER $\alpha$  protein expression in the range 10 nM to 1  $\mu$ M, whereas gene expression was induced at 1 and 10  $\mu$ M and repressed at 25  $\mu$ M (Lei, Tang, et al. 2021). In mouse 3T3-L1 adipocytes, TBBPA induced the gene expression of ER $\alpha$  only at 1 pM, of ER $\beta$  up to 10  $\mu$ M and of ERR $\gamma$  at 100 pM (Chappell et al. 2018). No evidence on modulation of ER $\alpha$  (except TCBPA), ER $\beta$  or ERR $\gamma$  gene/protein expression by TCBPA, BPE, BPP, BPZ, BPAP, and BPS-MAE is available.

Effects on the membrane G protein-coupled ER (GPER, or GPR30), mediating non-genomic estrogen signaling and recognized as a BPA target (Cimmino et al. 2019) were assessed only for the halogenated BPA alternatives. In particular, proliferation of ovarian OVCAR-3 and granulosa KGN cells was promoted by TBBPA through the GPER pathway, whereas TCBPA did not affect cell growth (Hoffmann et al. 2017). In same cells, both compounds did not affect GPER gene expression (Hoffmann et al. 2017). In mouse MA-10 Leydig cells, both BPA and TCBPA (1 nM) repressed GPER protein expression, whereas TBBPA (10 nM) significantly induced it (Gorowska-Wojtowicz et al. 2019). However, in breast cell lines, TCBPA induced GPER gene and protein expression (Lei, Tang, et al. 2021; Yu M et al. 2023).

In summary, despite the difference in the methods and cellular models used, the collected evidence highlights that the two halogenated compounds are very different in their estrogenic activity, with TBBPA being almost inactive and TCBPA having agonist activity toward both ERs. The non-halogenated BPA alternatives displayed a spectrum of different activities: BPE and BPZ had the highest agonist potency toward all the ERs, including ERR $\gamma$ , whereas BPAP had a moderate activity; BPAP seemed more active for ER $\beta$  and BPZ for ER $\alpha$ ; BPP had a stronger antagonist activity for both ERs and no activity for ERR $\gamma$ . BPS-MAE appeared to be inactive but more data are needed to substantiate this evidence.

### 3.2.2. Androgen receptor (AR)

BPA is known to antagonize AR (Rubin 2011). TBBPA dose-dependently displaced testosterone from AR (Beck et al.



2016). BPP and BPZ had a moderate and low activity, respectively, whereas BPA and BPE were not active (Liu X et al. 2019). No data on binding of TCBPA, BPAP, and BPS-MAE are available.

Different transactivation assays were performed to evaluate agonist/antagonist activity of BPA alternatives and none of them evidenced agonistic activity for AR. Contrasting evidence is available for halogenated derivatives as regards their anti-androgenic activity. In some cells stably transfected with AR, no activity was detected for TCBPA and TBBPA (Kitamura et al. 2005; Molina-Molina et al. 2013). TBBPA was not active also in AR-CALUX and two-hybrid yeast assays (Hamers et al. 2006; Li J et al. 2010). Opposite evidence was reported for TCBPA displaying anti-androgenic activity in a gene reporter assay on kidney fibroblast CV-1 cells in presence of 5 $\alpha$ -dihydrotestosterone (DHT) 1 nM (Sun et al. 2006), or in a two-hybrid AR yeast assay (Li J et al. 2010). TBBPA antagonism was observed with a yeast receptor bioassay (Roelofs et al. 2015), and in transfected HEK-293 cells with TBBPA (10  $\mu$ M) reducing AR activation by 37% in presence of testosterone (Beck et al. 2016).

In transfected HELN cells, BPA, BPE, BPZ, BPP, TCBPA, and BPAP, but not TBBPA, displayed AR antagonism, with different potencies (Grimaldi et al. 2019). BPE had a stronger antagonistic effect than BPA in various reporter or yeast assays (Rosenmai et al. 2014; Conroy-Ben et al. 2018; Šauer et al. 2021). In transfected Chinese hamster ovary (CHO)-1 cells, the following anti-androgenic potency was found: BPE > BPA > BPZ, BPP, and BPAP (Kojima et al. 2019); BPZ failed to show antagonism in a yeast assay (Conroy-Ben et al. 2018). Conversely, in transfected HepG2 cells, BPA, BPZ, and BPE had antagonistic effects with comparable high potencies; BPP was less potent, whereas BPAP and BPS-MAE did not show any significant activity (Pelch KE et al. 2019). BPE, BPZ, BPP, and BPAP showed anti-androgenic effects also in transfected MDA-kb2 mammary cells (Kolšek et al. 2015; Ma M et al. 2022), whereas in an AR-CALUX assay BPP did not show significant anti-androgenic activity (Šauer et al. 2021). BPS-MAE had no effect (Pelch KE et al. 2019).

TCBPA and TBBPA did not affect proliferation of MCF-7 cells transfected with AR (Molina-Molina et al. 2013). No evidence is available for BPE, BPP, BPZ, BPAP, and BPS-MAE. BPAP at 10  $\mu$ M decreased AR protein nuclear expression in 22Rv1, a prostate cell line, as well as in MCF-7/LLC2 cells in presence of DHT, but not in prostate LNCaP cells. Similarly, BPAP decreased AR gene expression and cell growth in 22Rv1 cells (Stossi et al. 2016).

Overall, TCBPA and TBBPA are not strong AR antagonists, and the following potency can be summarized for the other alternatives BPE > BPZ > BPP > BPAP; BPS-MAE is inactive, but this finding is supported by only one study.

### 3.2.3. Progesterone receptor (PR)

BPP and BPAP showed moderate binding activity toward PR, BPZ was very weak and BPE completely inactive, like BPA (Liu X et al. 2019). No report on TCBPA, TBBPA, and BPA-MAE binding affinity is available.

No agonist activity of BPA and its alternatives toward PR was evidenced in transfected HELN-PR cells. Rather, BPP, BPZ, and BPAP displayed strong antagonistic activity while BPE, TCBPA, and TBBPA were inactive as BPA (Grimaldi et al. 2019). In PR-CALUX assays, TBBPA, BPE, and BPP did not show antagonist activity (Hamers et al. 2006; Šauer et al. 2021). Conversely, TCBPA and TBBPA displayed some antagonist activity in a two-hybrid yeast assay (Li J et al. 2010).

In MCF-7 cells, TCBPA induced PR gene expression in the range 10 nM to 1  $\mu$ M (Lei, Tang, et al. 2021) or protein expression at 10  $\mu$ M, to an extent comparable to BPA 300  $\mu$ M, while TBBPA 30  $\mu$ M had a lower effect (Samuelson et al. 2001; Olsen et al. 2003). On the contrary, in breast SKBR3 cells, TCBPA repressed PR gene expression in the range 10 nM to 10  $\mu$ M (Yu M et al. 2023). In another study on MCF-7 cells, TCBPA did not affect PR gene expression at 1  $\mu$ M; otherwise, at same concentration, BPA, BPZ, BPE, BPP, and BPAP all induced PR gene expression (Pelch KE et al. 2019).

Overall, no definitive conclusion could be drawn for TCBPA, TBBPA, BPP, BPZ, and BPA on their interaction with PR due to the contrasting evidence. BPE is the only compound appearing quite inactive. No evidence is available for BPS-MAE.

### 3.2.4. Glucocorticoid receptor (GR)

In two different competitive binding assays, TBBPA had no affinity (Beck et al. 2016) or strong affinity for GR (Liu QS et al. 2020); BPP and BPZ had a strong binding affinity for GR, fivefold higher than BPA, whereas BPAP was moderately active (Liu X et al. 2019). No data on binding affinity is available for TCBPA, BPE, and BPS-MAE.

In transfected HMLN-GR cells, BPP was a potent GR antagonist while BPA, BPZ, BPAP, BPE, TCBPA, and TBBPA had no activity (Grimaldi et al. 2019). Similarly, TBBPA was inactive in two transfected models (Beck et al. 2016; Chappell et al. 2018). However, by using a yeast assay, TBBPA displayed a strong GR antagonism (Roelofs et al. 2015) and in transfected CHO-K1 cells, BPA, BPAP, BPP, and BPZ were all antagonists and BPE inactive (Kojima et al. 2019). BPAP may exert GR agonist activity in MDA-kb2 cells, whereas BPA, BPP, and BPZ were agonist in MDA-kb2 and CHO-K1 cells; in addition, BPA was antagonist in both cells whereas BPP and BPZ were antagonist in CHO-K1 and MDA-kb2 cells, respectively. BPE confirmed to have no activity on GR (Chen Q et al. 2020). However, in studies using transfected MDA-kb2 cells, BPE exerted GR agonism while BPA, BPP, BPAP, and BPZ were antagonists (Kolšek et al. 2015; Ma M et al. 2022).

In one report using 3T3-L1 cells, TBBPA induced GR gene expression (Chappell et al. 2018). No evidence is available for the other BPA alternatives.

In summary, TCBPA seemed to be inactive but was investigated in only one report. Also, TBBPA and BPE appeared to have no clear activity toward GR. Evidence supports BPP and BPZ as GR antagonists whereas contrasting findings are available for BPAP. No data are available for BPS-MAE.

**Table 3.** Summary of the range of active concentrations reported in the literature for each BPA alternative, grouped by *in vitro* assay categories as regard thyroid-disrupting properties.

	BPAP	BPE	BPP	BPS-MAE	BPZ	TBBPA	TCBPA
TR $\alpha$ binding affinity						IC <sub>20</sub> 1.5 $\mu$ M (RBP/T3 = 0.07)	
TR $\alpha$ gene reporter assay							
Agonism		Potency 70 $\mu$ M				10–60 $\mu$ M 10–100 $\mu$ M	NA/weak agonist
Antagonism							100 $\mu$ M
GH3 cell proliferation	$\nearrow$ 1 $\mu$ M		$\nearrow$ 1 $\mu$ M		$\nearrow$ 1 $\mu$ M	$\nearrow$ 1 $\mu$ M $\searrow$ 100 $\mu$ M	$\nearrow$ 10–100 $\mu$ M
THR $\beta$ binding affinity						IC <sub>20</sub> 5 $\mu$ M (RBP/T3 = 0.01)	
THR $\beta$ gene reporter assay							
Agonism		NA					
Antagonism							
Expression of TH-target genes <i>in vitro</i> (GH3 cells)							
Tsh $\beta$	NA		NA		NA		
Thrx	$\searrow$ (34 $\mu$ M)		NA		$\searrow$ (0.37 $\mu$ M)		
Thrb	$\searrow$ (34 $\mu$ M)		$\searrow$ (29 $\mu$ M)		$\searrow$ (3.7 $\mu$ M)		
Dio1	NA		$\searrow$ (29 $\mu$ M)		NA		
Dio2	$\searrow$ (34 $\mu$ M)		$\searrow$ (29 $\mu$ M)		$\searrow$ (37 $\mu$ M)		
TTR binding		$\searrow$ T4 binding IC <sub>50</sub> 0.13 mM				$\searrow$ T4 binding IC <sub>50</sub> 7.7 nM (RBP/T4 1.5–10.6)	$\searrow$ T4 binding IC <sub>50</sub> 107 nM (RBP/T4 0.76)
TBG binding						NA	NA

NA: not active.

Values related to genes/protein expression are concentrations at which significant effects were observed. The detailed list of all the assays and the related publications is shown in Table A2. When given in the article, relative binding potencies (RBP) toward the natural ligand (T3 for THR, T4 for TTR) are reported. Effects on the expression on TH synthesis-related genes are not reported as they rely on a single study (Lee S et al. 2017) and did not show an effect of the tested compounds (BAP, BPP, and BPZ).

### 3.2.5. Peroxisome proliferator activated receptors (PPAR $\alpha$ , $\beta/\delta$ , $\gamma$ )

By using a whole-cell competitive assay or affinity columns, both TCBPA and TBBPA, but not BPA, showed to be strong binders of PPAR $\gamma$  (Riu, Grimaldi, et al. 2011; Riu, Le Maire, et al. 2011). TBBPA confirmed to have a high affinity for PPAR $\gamma$  (Liu QS et al. 2020), whereas BPA, BPE, BPP, BPZ, and BPAP were inert or inactive (Liu X et al. 2019). Both TBBPA and TCBPA exhibited about 18- and 12-fold stronger binding affinity than BPA to PPAR $\beta/\delta$ , respectively (Li CH et al. 2021). The other BPA alternatives were not investigated for their binding to PPAR $\beta/\delta$  and no compound was evaluated for PPAR $\alpha$  affinity.

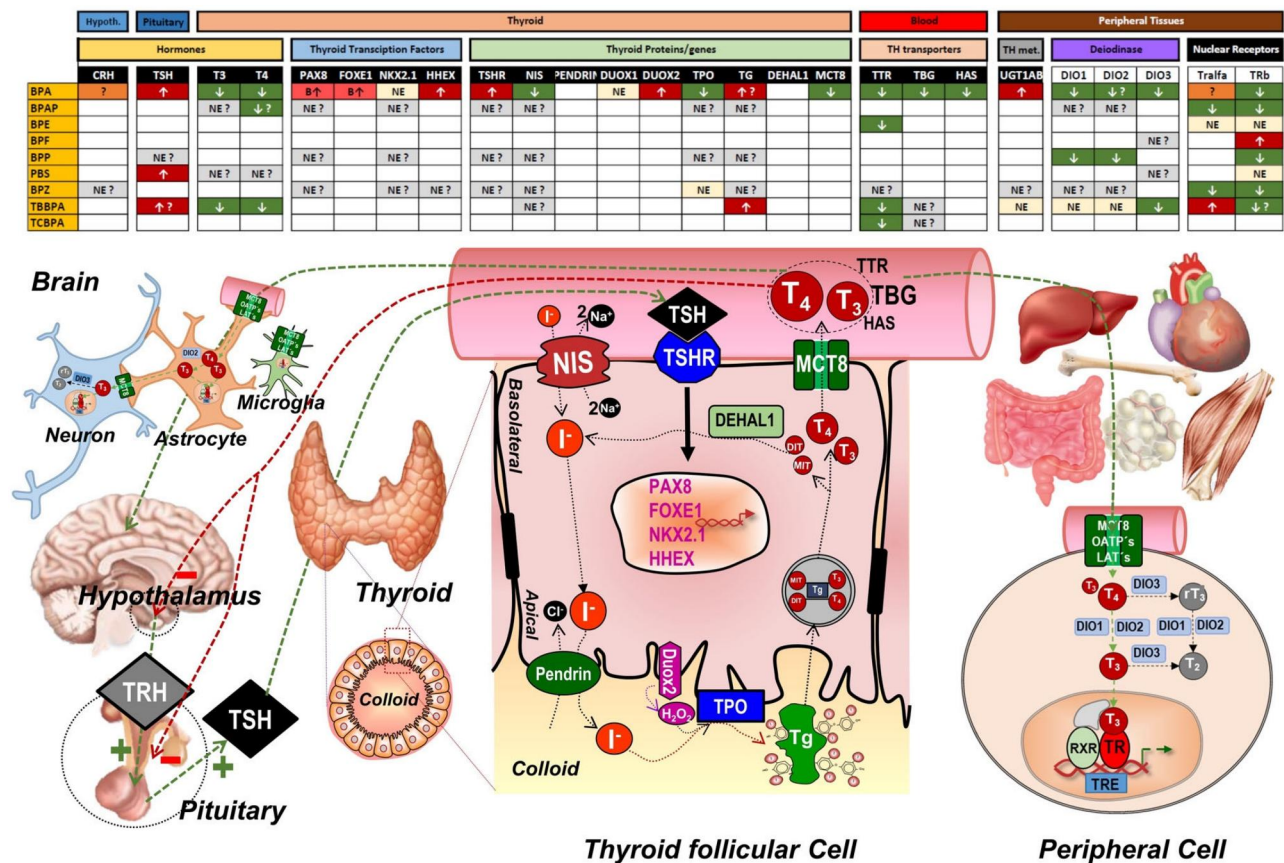
TCBPA and TBBPA exhibited a high potency in activating PPAR $\gamma$  gene expression in transfected cells (Riu, Grimaldi, et al. 2011; Garoche et al. 2021). In addition, TBBPA had agonist activity in cells transfected with mouse or human PPAR $\gamma$  (Akiyama et al. 2015; Watt and Schlezinger 2015; Chappell et al. 2018; Andrews et al. 2020; Liu QS et al. 2020). TCBPA and TBBPA showed agonistic activity also for PPAR $\beta/\delta$  (Li CH et al. 2021), whereas they did not activate PPAR $\alpha$  and PPAR $\beta$  using other reporter assays (Riu, Grimaldi, et al. 2011; Riu, Le Maire, et al. 2011). The other alternatives were not investigated for their capability to trans-activate PPARs, most probably for their lack of binding affinity, at least for PPAR $\gamma$ .

TBBPA induced PPAR $\gamma$  gene and/or protein expression in several *in vitro* models (Akiyama et al. 2015; Honkisz and Wójtowicz 2015b; Watt and Schlezinger 2015; Chappell et al. 2018; Kakutani et al. 2018; Liu QS et al. 2020; Kim S et al.

2021; Cheng and Volz 2022). In MA-10 mouse Leydig cells, TBBPA (10 nM) significantly induced PPAR $\gamma$  protein expression unaffected PPAR $\beta$ , whereas TCBPA at 1 nM significantly decreased the protein expression of PPAR $\beta$  and PPAR $\gamma$ . Both increased the expression of PPAR $\alpha$ , TCBPA to a higher extent (Gorowska-Wojtowicz et al. 2019). TBBPA also up-regulated PPAR $\alpha$  gene expression in 3T3-L1 mouse adipocytes (Kim S et al. 2021) and PPAR $\beta/\delta$  transcript levels in steatotic FaO rat hepatoma cells while decreasing the expression of PPAR $\gamma$  and  $\alpha$  (Grasselli et al. 2014) in these cells. A drop in PPAR $\gamma$  protein expression was also induced by TBBPA in mouse neocortical neurons (Wojtowicz et al. 2014), whereas it did not affect PPAR $\gamma$  gene expression in human bone marrow cells (Andrews et al. 2020). Overall, the available evidence points to TBBPA as the only BPA alternative exerting a strong PPAR $\gamma$  agonism, with cell type-dependent effect on PPAR $\gamma$  expression.

### 3.2.6. Other receptors

Some studies were performed to explore interaction of BPA alternatives with receptors involved in the metabolism of xenobiotics. BPAP, BPP, and BPZ had a strong binding affinity for the pregnane X receptor (PXR), higher than that of BPA, whereas BPE was inactive (Liu X et al. 2019). In transfected HepG2 cells, TCBPA and TBBPA did not activate human and mouse PXR conversely to BPA which activated human PXR (Sui et al. 2012); however, by using transfected HG5LN-PXR cells, both TCBPA and TBBPA displayed agonistic activity similarly to BPA (Molina-Molina et al. 2013). In same cells, TCBPA,



**Figure 1.** Pathways related to thyroid hormone (TH) metabolism disruption by BPA and alternatives. Regulation of TH synthesis starts with the production of TRH in the hypothalamus that activates TSH production in the pituitary gland. TSH is the main regulator of thyroid follicular cells through the TSHR that activates several nuclear transcription factor (PAX8, FOXE1, NKX2.1, and HHEX), that subsequently activate the expression of genes involved in thyroid differentiation (Fernández et al. 2015). Dietary iodide ( $I^-$ ) enters the follicular cell through NIS, localized at the basolateral plasma membrane (De la Vieja and Santisteban 2018; Koumariou et al. 2022). Later  $I^-$  crosses the apical membrane, mainly through PENDRIN, into the colloid where TPO, using  $H_2O_2$  produced by DUOX2, oxidizes the tyrosine residues of TG to form different iodine-containing compounds (MIT, DIT, T3, and T4) (Di Jeso and Arvan 2016). The amount of T4 synthesized is much higher than that of T3. Under metabolic requirement of TH, TG is endocytosed into the follicular cell and the iodine-compound is enzymatically released. DEHAL1 enzyme discharges  $I^-$  from inactive iodide-containing compounds (MIT and DIT) to be reincorporated in the TH synthesis process (De la Vieja and Santisteban 2018; Riesco-Eizaguirre et al. 2021). THs (T3 and T4) are transported to the bloodstream mainly through MCT8 where they are bound to TH transporters (TBG, TTR, and HAS) and delivered to different peripheral tissues (Mimoto and Refetoff 2020). TH is transported to peripheral cell cytoplasm by different transporters (MCT8, MCT10, LAT1, LAT2, OATP1C1, OATP2B1, and OATP3A1) in a tissue-specific manner (Bernal et al. 2015). Iodothyronine deiodinases DIO1 and DIO2 in cell cytoplasm remove one  $I^-$  from the inactive TH T4 to convert it to the active TH T3, that can be inactivated to T2 by removing an additional  $I^-$  by DIO3 (Darras and Van Herck 2012). T3 enters the nucleus where it activates the expression of a large number of genes in a tissue-specific manner by engaging TR and forming a complex with retinoid X receptor (RXR) and other co-activators/repressors that bind thyroid hormone response elements (TRE) located in the regulatory regions of target genes (Vella and Hollenberg 2017). THs also regulate their own synthesis by inhibiting the production of TRH and TSH in the hypothalamus and pituitary, respectively (Larsen 1982). THs enter the brain either directly via the blood-brain barrier (BBB) or indirectly via the blood-CSF-barrier (B-CSF-B) where MCT8 and OATP1C1 are essential (Bernal et al. 2015). The disruption of TH metabolism by BPA has been studied at different levels and in different *in vivo* and *in vitro* models. As can be seen in the top table, most of the genes/proteins essential in this process have been studied (with the exception of PENDRIN and DEHAL1). However, in the case of the remaining BPA analogues numerous gaps (blank table cells) remain. Furthermore, in many cases, an apparent lack of analogue effect (indicated as "NE?") was observed, although this could be due to low tested concentrations (see article). TH: thyroid hormones; TRH: thyrotropin-releasing hormone; TSH: thyroid-stimulating hormone; TSHR: TSH receptor; PAX8: paired-box gene 8; FOXE1: forkhead box protein E1; NKX2.1: NK homeobox 1; HHEX: hematopoietically expressed homeobox 1; NIS: sodium/iodide symporter; pendrin, anion exchange protein encoded by SLC6A4 gene; TPO: thyroid peroxidase; DUOX2: dual oxidase 2; TG: thyroglobulin; MIT: monoiodotyrosine; DIT: diiodotyrosine; DEHAL1: iodotyrosine dehalogenase 1; TBG: thyroxine-binding globulin; TTR: transthyretin; HAS: human serum albumin; MCT8/10: monocarboxylate transporter 8/10; LAT1/1: large neutral amino acids transporter small subunit 1/2; OATPs: organic amphipathic transporters; DIO1/2/3: type 1/2/3 iodothyronine deiodinase; TR: nuclear thyroid hormone receptor; NE: non-effect; ↑: increasing or upregulation; ↓: decreasing or downregulation; B↑/↓: biphasic up/down-regulation; The "?" symbol means that there are insufficient or contradictory results.

BPZ, and BPP were strong PXR agonists, BPA, BPAP, and TBBPA were moderate agonists, whereas BPE was inactive (Grimaldi et al. 2019). Similarly, in transfected COS-7 cells, BPAP and BPZ were strong agonists, BPA and BPP were less potent and BPE had no activity (Kojima et al. 2019).

Concerning other receptors, the literature is very limited and thereby the following available data are not included in Table A1 and Table 2. Among the receptors involved in detoxification of xenobiotics, the aryl hydrocarbon receptor

(AhR) is particularly relevant, since it shares similar functionality with them, although, not belonging to the NR family (Mackowiak and Wang 2016). TBBPA was inactive in a DR-CALUX assay (Hamers et al. 2006), whereas in a reporter gene assay BPE displayed agonistic effect toward AhR with a potency higher than that of BPA (Rosenmai et al. 2014).

BPE, BPAP, and BPZ displayed a strong binding affinity for the constitutive androstane receptor (CAR), lower than BPA, whereas BPP was inactive (Liu X et al. 2019). No BPA



alternative was a CAR agonist, rather all of them were antagonists with the following potency order BPZ > BPAP > BPA, BPP, and BPE being inactive (Kojima et al. 2019).

In competitive binding assays, BPA, BPAP, BPE, BPP, and BPZ were inert toward retinoic acid receptors (RAR $\alpha$ , RAR $\beta$ , and RAR $\gamma$ ), and RAR-related orphan receptors (ROR $\alpha$ , ROR $\beta$ , and ROR $\gamma$ ). Only BPP had very weak activity for retinoid X receptors (RXR $\alpha$ , RXR $\beta$ , and RXR $\gamma$ ) (Liu X et al. 2019). However, BPAP, BPE, BPZ, and BPP were previously shown not to bind ROR $\gamma$ , as BPA (Nishigori et al. 2012). In a reporter gene assay, TBBPA had no agonistic activity for RAR $\alpha$ , but displayed a strong antagonism, higher than BPA (Xu H et al. 2022). In addition, TBBPA had no activity for hRXR $\alpha$  in transfected 3T3-L1 cells; however, *RXR $\alpha$*  gene expression was increased in same cells by TBBPA at 1 pM and 10  $\mu$ M (Chappell et al. 2018). Conversely, TCBPA displayed antagonist activity for RXR $\beta$  over 7-fold higher than BPA (Li N et al. 2016).

BPA, BPAP, BPE, and BPZ were inert toward the liver X receptors (LXR $\alpha$ , LXR $\beta$ ) as well as the vitamin D receptor (VDR), whereas BPP was inactive toward VDR but had strong binding affinity for both LXRs (Liu X et al. 2019). TCBPA and TBBPA exhibited a binding potency 8.7- and 34-fold higher than BPA, respectively, for the farnesoid X receptor (FXR). However, their transactivation activity was lower than BPA, significantly inducing FXR expression only at the highest concentrations 25–50  $\mu$ M (Zhang et al. 2023).

No compound showed agonist activity toward the mineralocorticoid receptor (MR); conversely, BPZ, BPAP, BPE, BPP, TCBPA, and TBBPA were all strong antagonists comparable to BPA (Grimaldi et al. 2019).

Overall, the evidence related to these receptors is too limited to draw conclusions on the activity of BPA alternatives. Further, BPS-MAE was never investigated. More studies are thus needed to clearly assess their ED potential considering all the possible receptor pathways.

### 3.2.7. Concluding considerations

Overall, a lot of data gaps exists on the ED activity of the prioritized BPA alternatives, especially on BPS-MAE, still poorly investigated. Importantly, the reported results reflect only the activity of parent compounds since no information is available on the metabolic competence of the cell lines used and the activity of the potential metabolites, with consequent biases in the qualitative and quantitative aspects of effect estimation.

## 3.3. Effects on thyroid hormone system pathways

Even though there is ample evidence of potential TH system disruption by BPA (see Table A2), large data gaps remain regarding the potential effects of BPA alternatives (Table 3). In rats, BPA effects on different parameters of the thyroid function have been more consistently associated with high doses (>40 mg/kg/bw per day), which are far above the highest estimated BPA human dietary intake (infants and toddlers up to 0.875  $\mu$ g/kg/bw per day; adult men and women up to 0.388  $\mu$ g/kg/bw per day; adolescents up to 1.449  $\mu$ g/kg/bw

per day), even after applying a HED factor of 0.068, according to EFSA criteria (EFSA 2015). All levels of regulation of the thyroid system and/or thyroid-related physiological processes are potential targets of bisphenols (Figure 1, Table 3, and Table A2). In this respect, TH receptors are discussed here in the context of TH system disruption.

### 3.3.1. Effects on TH levels

Animal studies linking BPA exposure to TH levels have shown variable results, with TH levels increasing (Zoeller et al. 2005; da Silva et al. 2019), decreasing (Viguié et al. 2013; Ahmed 2016; Jiang et al. 2016; Guignard et al. 2017; Silva BS et al. 2019; Mohammed et al. 2020), or not changing (Kobayashi et al. 2005; Sadowski et al. 2014; Bansal and Zoeller 2019). In relation to this variability, studies showed biphasic responses and/or responses depending on sex, age, exposure route, and duration (Xu X et al. 2007; Santos-Silva et al. 2018). Among the BPA alternatives into focus in this review, data on TH levels are available only for TBBPA. All the related studies (Van der Ven et al. 2008; Saegusa et al. 2009; EFSA 2011; Cope et al. 2015; Osimitz et al. 2016; Sanders et al. 2016), but one (Meerts et al. 1999), consistently showed a decrease in T4 concentrations. Results on T3 or thyroid-stimulating hormone (TSH) are less consistent.

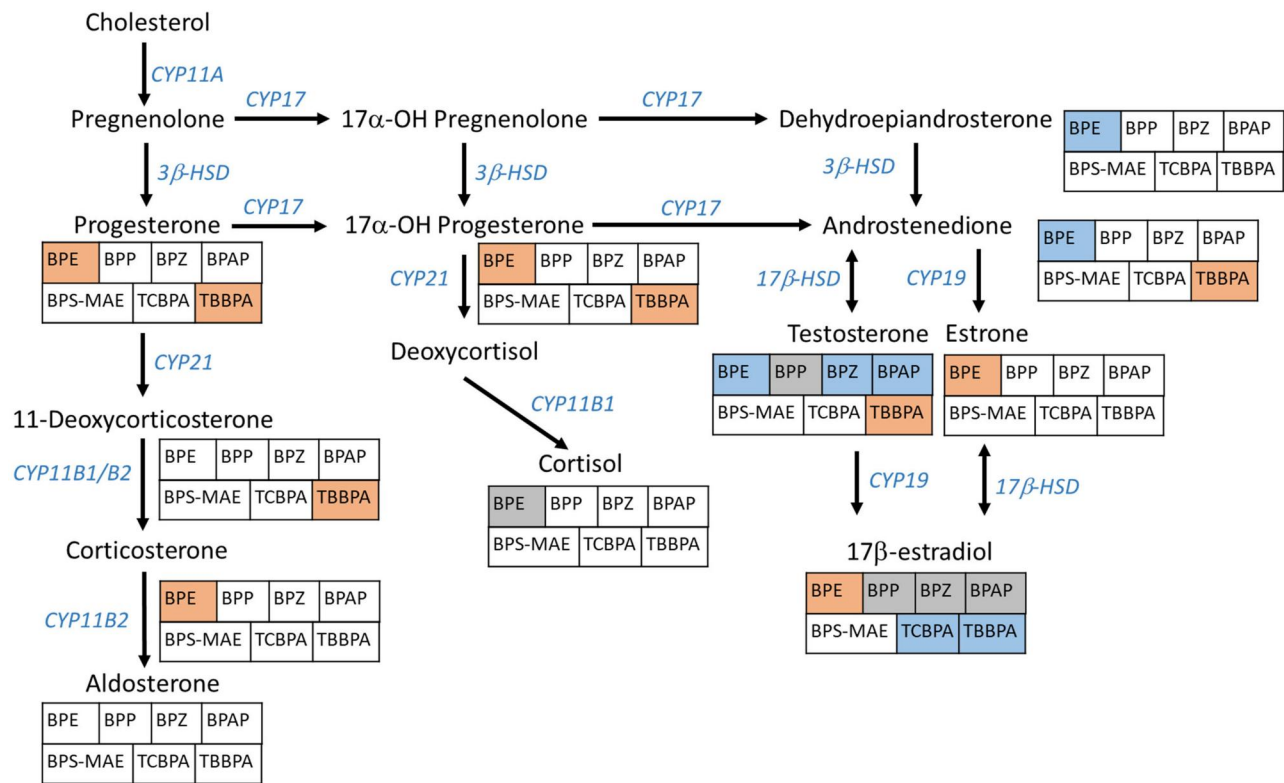
The paucity of basic data on the effect of BPA alternatives on TH levels *in vivo* highlights the huge gaps and needs for data to characterize the effects and modes of action of these molecules on the thyroid system.

### 3.3.2. Effects on thyroid hormone receptors (THR $\alpha$ )

BPA is a weak ligand of THR $\alpha$ , inhibiting T3-induced response in *Thrx* and *Thrb* gene reporter assays in a dose-dependent manner (Moriyama et al. 2002). A structure-based study indicated that BPA analogues, including BPP, BPZ, and BPAP could form important polar and hydrophobic interactions with several THR $\alpha$  residues, most of which also bind the native ligand T<sub>3</sub> (Beg and Sheikh 2020). In a fluorescence competitive binding assay, TBBPA displayed relative binding potencies to THR $\alpha$  and  $\beta$  about 15- and 100-fold lower than that of T<sub>3</sub>, respectively (Ren et al. 2020).

Different gene reporter assays evidenced modest transactivation potency for THR $\alpha$  of TBBPA, BPA, and BPE from 10 to 60  $\mu$ M, 30 and 70  $\mu$ M for TBBPA, BPA, and BPE, respectively *i.e.* much lower than that of T<sub>3</sub>, while TCBPA showed low or no activity (Jugan et al. 2007; Lei et al. 2017). However, in a yeast two-hybrid assay incorporating rat *Thrx*, TBBPA, and TCBPA displayed agonist activity (Terasaki et al. 2011). In rat GH3 cell line, BPA, BPAP, BPP, BPZ, TBBPA, and TCBPA all showed a positive effect on GH3 cell proliferation suggestive of THR agonism (Kitamura et al. 2002; Lee J et al. 2018). For TBBPA and TCBPA, however, antagonistic effects on T3-mediated responses were also shown (Jugan et al. 2007).

Neither BPA nor BPAF (10<sup>-9</sup> to 10<sup>-5</sup> M) appeared to influence *Thr* expression in transfected human granulosa cell line COV434 (Mlynarcikova and Scsukova 2020). By contrast, in rat GH3 cell lines, BPA and BPAP decreased *Thrx* and *Thrb* expression at 44 and 34  $\mu$ M, respectively (Lee S et al. 2017).



**Figure 2.** Schematic representation of the available evidence on effects exerted by BPA alternatives on steroidogenesis from *in vitro* studies in human H295R cells and mouse MA10 Leydig cells. Detailed data are provided in Table A3. Colored boxes indicate induction (orange), inhibition (blue), or inactivity (grey). White boxes indicate that the compounds were not assessed. Steroidogenic enzymes are indicated in blue italics. CYP: cytochrome P450; HSD: hydroxysteroid hydrogenase.

Furthermore, BPP had no effect on *Thrx* expression within the range of 30 nM to 30 μM, while it inhibited *Thrb* expression from 3 μM in the same cell line. BPZ decreased *Thrx* and *Thrb* expression in a concentration-dependent manner from 0.37 to 3.7 μM for *Thrx* and *Thrb*, respectively (Lee S et al. 2017).

BPA, at low concentrations (nM), might negatively regulate THR transcriptional activity via an indirect mechanism involving the recruitment of nuclear receptor corepressor (N-CoR) or silencing mediator for retinoid and thyroid-hormone receptor (SMRT) to THRβ1 (Moriyama et al. 2002; Sheng et al. 2012). No data are currently available suggesting that other bisphenols might trigger such mechanisms.

Overall, evidence on halogenated derivatives of BPA suggests they may exert some agonistic effects on THRs. BPE showed some transactivation activity only toward THRα and indirect evidence of some agonism is available for BPAP and BPP in relation to cell proliferation. However, data on gene expression modulation of THRs mostly indicate an inhibitory effect of these compounds; therefore, more studies are needed to clarify this contrasting evidence. In addition, to our knowledge, information is lacking for BPS-MAE.

### 3.3.3. Effects on TH biosynthesis

*In vivo* data, mostly obtained with high to very high doses (20 up to 400 mg/kg/d), are suggestive of inhibitory effects of BPA and/or TBBPA on TH biosynthesis whatever the evaluated parameter: thyroid histology, gene expression, iodine uptake, and TPO activity (da Silva et al. 2018; Mohammed

et al. 2020; Hu C et al. 2023). In FRTL5 rat thyroid cells, BPA (100 μM) decreased *Slc5a5* and *Tpo* while increasing *Tg* (from 30 μM), *Pax8* and *Foxe1* (100 μM) expressions (Wu Y et al. 2016). This was correlated with a noncompetitive inhibition of iodine capture by BPA at high concentration (100 μM) while no impact on TPO activity could be shown (Wu Y et al. 2016). Interestingly, in FRTL-5 cells treated with BPA, a biphasic response was observed for *Pax8* and *Foxe1* expression (Gentilcore et al. 2013). BPP, BPZ, or TBBPA had no effect on *Dio1*, *Dio2*, *Tshβ*, *Tshr*, *Slc5a5*, *Nkx2.1*, *Pax8*, *Tg*, and *Tpo* expressions (Lee S et al. 2017). In this same study, BPA and BPAP seemed to positively regulate the transcription of *Slc5a5* at quite high concentrations of 0.4 mM and 34 μM for BPA and BPAP, respectively. Interestingly, in PCCL3 rat thyroid cell, BPA at low concentration (10 nM) decreased *Tpo* and *Slc5a5* mRNA levels (da Silva et al. 2018). BPA can inhibit *Tshβ* expression in adult female rats at very high dose (da Silva et al. 2018) and in GH3 cells at high concentration (Lee S et al. 2017). BPAP and BPZ had no effect on *Tshβ* expression in GH3 cells (Lee S et al. 2017).

In conclusion, most of the BPA alternatives studied seemed to not affect TH biosynthesis pathways, except TBBPA, which decreased the expression of some related genes in mice and BPAP, which was able to induce *Slc5a5* gene expression in an *in vitro* model. No data are available for TCBPA, BPE, and BPS-MAE; thus, further studies are warranted to complete the information on all the prioritized BPA alternatives.



### 3.3.4. Interaction with TH serum transporters

Some studies showed that BPA can bind to serum TH transporters such as human serum albumin (HSA), thyroxine-binding globulin (TBG), and transthyretin (TTR) (Cao et al. 2011), although this seemed to be concentration-dependent, with little binding activity in the nM range (Meerts et al. 2000; Marchesini et al. 2008). All data on BPA binding capacities to TTR agreed on showing a very low potency of BPA to displace TH from TTR (Yamauchi et al. 2003; Kudo et al. 2006; Šauer et al. 2021). By contrast, available data indicate that TBBPA and TCBPA exhibited much higher binding capacities (Yamauchi et al. 2003; Kudo et al. 2006; Šauer et al. 2021). In particular, TBBPA had a relative potency about 10-fold higher than that of T4 to displace labeled-T4 from TTR, whereas TCBPA was very similar to T4 (Meerts et al. 2000). BPE behaved similarly to BPA toward T4 binding to TTR (Šauer et al. 2021). A bioinformatics study showed that BPP and BPAP should be able to form polar and hydrophobic interaction with the native T4 binding residues in TBG (77–100%) (Beg and Sheikh 2020).

Overall, although some evidence exists on the binding activity of some BPA alternatives to TH serum transporters no experimental reports related to TTR binding are available for BPZ, BPP, BPAP, and BPS-MAE. In addition, information is lacking for the complete panel of prioritized BPA alternatives as regards binding to HSA and TBG.

### 3.3.5. Interaction with TH plasma membrane transporters

The transport of TH into or out of cells is critical in TH-related processes, not only in the thyroid, but also in all target tissues and especially at the level of the placenta during pregnancy and in the brain in stages of embryonic and fetal development (Bernal et al. 2015). An impact of BPA on MCT8-mediated T3 uptake was shown on MDCK cells overexpressing human MCT8 (Dong and Wade 2017) but at very high concentration (250  $\mu$ M) at the limit of cytotoxicity. This was not confirmed at lower concentrations on primary mouse astrocytes or MCT-8 transfected cells (Johannes et al. 2016). To the best of our knowledge, there are no available data evaluating the effect of BPZ, BPE, BPP, BPAP, PBS-MAE, TCBPA, and TBBPA on MCT8-mediated TH uptake.

### 3.3.6. Concluding considerations

To summarize, from the review of the numerous experimental data available on the effects of BPA on the thyroid-related pathways, it can be legitimately hypothesized that BPA is not neutral on thyroid regulation. For BPA alternatives, because of the paucity of available data (for summary see Table 3), many gaps remain that should be addressed in a well-structured frame with validated assays and with dose ranges relevant to human exposure. Our analysis highlights in particular the lack of "functional endpoints" such as NIS or TPO activities.

## 3.4. Effects on steroidogenesis

Compared to the studies investigating interaction of BPA alternatives with NRs, effects on steroidogenesis are

somewhat underexplored. Available evidence is detailed in Table A3 and summarized in Figure 2.

Only two reports (Rosenmai et al. 2014; Lin et al. 2021) analyzed impairment of steroid hormone synthesis in the human adreno-carcinoma cell line H295R as per the OECD Test Guideline 456 (OECD 2023) performing complete dose-response assessments (Rosenmai et al. 2014), or evaluating effects of chemicals at three selected concentrations (Lin et al. 2021).

BPE induced both estrone and E2 secretion in H295R cells with  $EC_{50}$  values comparable to those of BPA (Rosenmai et al. 2014). While BPA did not affect progesterone secretion and decreased  $17\alpha$ -OH progesterone levels, BPE induced both progesterone and  $17\alpha$ -OH progesterone. This would support a different mechanism for BPA and BPE to affect E2 synthesis, even if both compounds decreased dehydroepiandrosterone (DHEA) and androstenedione, upstream the synthesis of sex steroids. Both BPA and BPE strongly reduced testosterone synthesis in H295R cells, with comparable potency. BPA decreased cortisol levels, in agreement with  $17\alpha$ -OH progesterone drop, and had no effect on corticosterone. On the contrary, BPE did not affect cortisol levels but increased corticosterone level, according to  $17\alpha$ -OH progesterone increase (Rosenmai et al. 2014).

BPP, BPZ, and BPAP did not affect E2 secretion in H295R cells, conversely to BPA (Lin et al. 2021). BPA significantly decreased testosterone secretion at 25 nM whereas BPAP decreased it at both 6.25 and 25 nM and BPP did not affect hormone levels. A non-monotonic dose-response was observed for BPZ, which induced a testosterone drop at 0.39 and 6.25 nM but not at 1.56 nM (Lin et al. 2021).

TBBPA did not affect E2 and testosterone levels at 0.025, 0.05, and 0.5  $\mu$ M in H295R cells (Song et al. 2008). In MA-10 mouse Leydig cells, BPA decreased E2 secretion at 1 nM; similarly, TCBPA 1 nM and TBBPA 10 nM decreased the secretion of E2, TCBPA to a higher extent (Gorowska-Wojtowicz et al. 2019). In same cells, estrone but not E2 was assessed, being unaffected by TBBPA at 10  $\mu$ M, which otherwise increased both progesterone and  $17\alpha$ -OH progesterone levels. TBBPA, at same concentration, also increased the synthesis of 11-deoxycorticosterone, androstenedione and testosterone. For testosterone, a dose-response increase was also observed, being significant at 30  $\mu$ M and 100  $\mu$ M, with a maximum 59-fold increase compared to control cells (Roelofs et al. 2015). A similar dose-response in MA-10 cells had been previously observed, with a 46-fold increase for TBBPA 30  $\mu$ M, compared to the sixfold induction by BPA (Dankers et al. 2013). E2 secretion was time- and dose-dependently increased by TBBPA in JEG-3 human choriocarcinoma placental cells. However, these cells are not able to synthesize E2 from cholesterol, thus effects were assessed in presence of the precursor DHEA (Honkisz and Wójtowicz 2015a). TBBPA also increased progesterone levels in JEG-3 cells with a similar time- and dose-dependency (Honkisz and Wójtowicz 2015b). In human placental explants, TBBPA significantly increased testosterone secretion and dose-dependently induced progesterone secretion, although with marginal significance only at the highest dose (range 5 nM to 50  $\mu$ M). Only at 5  $\mu$ M, a

significant drop in E2 production was reported (Arita et al. 2018), in contrast to what observed in JEG-3 cells.

Beside steroid hormones, TBBPA significantly decreased the human chorionic gonadotropin ( $\beta$ -hCG) production in JEG-3 cells at all time points (24–72 h) (Honkisz and Wójtowicz 2015b). Moreover, both TCBPA and TBBPA increased  $\beta$ -hCG protein expression in mouse MA-10 Leydig cells (Gorowska-Wojtowicz et al. 2019).

As regards enzymes involved in the steroidogenic pathway, BPZ significantly repressed *CYP11A* expression at 6.25 nM in H295R cells, and did not affect levels of *CYP17*, *CYP19*, steroidogenic acute regulatory protein (*StAR*) and  $3\beta$ -hydroxysteroid dehydrogenase type 2 (*3\beta*-HSD2). BPAP had no effect on any enzyme (Lin et al. 2021). In same cells, TBBPA had no effect on aromatase activity (*CYP19*) (Cantón et al. 2005), but induced *CYP21* expression (Song et al. 2008). TBBPA significantly increased *StAR*, *Cyp11A1*, and *Cyp17* expression in one study (Dankers et al. 2013) whereas no effect was observed in another report (Roelofs et al. 2015). Both studies confirmed the lack of effects on *3\beta*-Hsd1 and *17\beta*-Hsd3 expression (Dankers et al. 2013; Roelofs et al. 2015). Conversely, in JEG-3 placental cells, TBBPA induced a time- and dose-response increase in aromatase enzyme activity and protein expression (Honkisz and Wójtowicz 2015a).

#### 3.4.1. Concluding considerations

Overall, TBBPA was the most investigated analogue in relation to all hormones and genes of steroidogenesis. One report assessed BPE effects on all steps of steroidogenesis. TCBPA was assessed only for effects on E2 secretion in mouse cells, whereas BPP, BPZ, and BPAP were assessed only for effects on E2 and testosterone production and some steroidogenic enzyme expression. No evidence on BPS-MAE is available and thereby additional studies are needed to have comparable results for all BPA alternatives, especially using validated methods as the OECD Test Guideline 456.

## 4. Immunotoxicity of BPA alternatives

### 4.1. General considerations

Immunotoxicology studies the adverse effects on the immune system caused by exposure to various physical and chemical agents. This complex system includes immunocompetent cells and soluble factors with specialized roles, divided into specific adaptive immunity and nonspecific innate immunity. The characteristics of the immune system make it vulnerable to xenobiotics (Germolec et al. 2022). Damage to this system can be associated with a wide range of adverse and sometimes life-threatening events, including immunosuppression, inappropriate immunostimulation, hypersensitivity, and autoimmunity. These effects are typically assessed using *in vitro* assays and *in vivo* models in both laboratory animals and humans (Maddalon et al. 2023).

Once believed to work independently from each other, it is now clear that the endocrine and the immune systems are tightly interwoven (Manley et al. 2018). As a close link exists between the endocrine and the immune systems, and the

immune system is highly receptive to endocrine signals due to the expression of hormone receptors on immune cells, any endocrine active substance is likely to affect the immune response (Sabuz Vidal et al. 2021).

Increasing evidence indicates, especially following developmental exposure, an ability of BPA in possibly modulating immune responses and signaling pathways, which leads to a proinflammatory response by favoring the differential polarization of immune cells (e.g. decreased T regulatory cells, increased T helper (Th) 17 cells) and cytokine production profile to one consistent with proinflammation (Sharif et al. 2022). In the recent EFSA opinion on the reevaluation of the risks to public health related to the presence of BPA in foodstuffs, effect on Th17 cells in mice was identified as the critical effect (EFSA 2023); these cells are pivotal in cellular immune mechanisms and involved in the development of inflammatory conditions, including autoimmunity and lung inflammation. In addition, epidemiological studies have discussed potential associations between several autoimmune diseases and BPA exposure, including neuroinflammation in the context of multiple sclerosis, colitis in inflammatory bowel disease, nephritis in systemic lupus erythematosus, and insulinitis in type 1 diabetes mellitus (Sharif et al. 2022). However, diverging views on BPA proinflammatory effects exist and should be considered (BfR 2023; BfR and EFSA 2023). In addition, more reports discuss an increased propensity to develop allergy, and asthma after dietary and inhalation exposure to BPA (Robinson and Miller 2015; McDonough et al. 2021).

### 4.2. Information on the immunotoxicity of BPA alternatives

While there are many original studies and reviews on the immunotoxicity of BPA (Robinson and Miller 2015; Xu J et al. 2016; Aljadeff et al. 2018; Sabuz Vidal et al. 2021; Sharif et al. 2022), only few studies have addressed the effects of BPA alternatives on the immune system (McDonough et al. 2021). In this section, a review of data regarding the ability of BPA alternatives to perturb the immune system is presented.

There is some evidence of BPP effects on the immune system. Indeed, mice exposed for 5 or 9 weeks to BPP (0.3, 30, and 3000  $\mu$ g/kg bw/day) exhibited gut microbiota dysbiosis, together with the activation of lipopolysaccharide/nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway and inflammation (Ma N et al. 2023).

Effects on immune parameters were also investigated for BPAP. BPAP exposure (400  $\mu$ g/kg bw/day) induced hippocampal inflammation, microglial inflammation, and a higher number of splenic macrophages and activated dendritic cells in mice (Wu X et al. 2023). These effects were associated with increased anxiety state level as reported in Section 5.

No studies evaluating the effects of BPZ or BPE exposure on the immune system are available. However, as described in Section 3, BPZ is threefold more potent than BPA in agonizing ER $\alpha$  and BPE has a similar potency than BPA in inducing ER $\alpha$  (Pelch KE et al. 2019). Given the tight connection between the endocrine and immune systems, it

**Table 4.** *In vitro* studies reporting neural effects of BPA alternatives (BPAP, BPE, BPP, BPZ, and TCBPA).

Compounds	Dose range	Cell types	Analyses	Reference
BPA, BPAF, BPB, BPE, BPF, BPS, and BPZ	All substances at 1, 10, and 100 nM in 0.01% DMSO	(i) Human embryonic stem cells (hESCs) (ii) hESC differentiated into neural epithelium (iii) Neuron-like cells differentiated from adherent neuronal stem cells	Cytotoxicity severity was assessed for all targeted compounds based on the IC <sub>50</sub> calculation or estimation; BPAF was defined as the most cytotoxic chemical tested whereas BPF and BPS the less cytotoxic. The results pointed out that BPA and its analogues did induce neurogenesis impairment (neural epithelium generation) at early stages of neurogenesis but did not exclude that the latter could interfere with a later stage of neurodevelopment. Gene expression related to axon guidance process was not modified by the exposure of BPA and its analogues; normalized values of the total and maximum- neurite length were down-regulated by BPA in a dose dependent manner. A significant reduction of total neurite length was noted for 4 out of the 6 BPA analogues tested (BPS, BPF, BPA, or BPAF) at 1 nM; while only 100 nM of BPB, BPZ, or BPE triggered a noticeable decrease in the total length of neurite.	Liang X et al. (2020)
BPA, BPAP, BPBP, BPC, BPE, BPG, BPP, and BPPH	All substances at 1 nM, 100 nM and 10 μM for 3 h or 24 h.	IMR-32 neuroblastoma cell line	<i>Treatment for 24 h:</i> All bisphenols except BPPH increased ROS levels, since 1 nM (BPA, BPE) or 100 nM (BPC) or at 10 μM (BPG, BPAP, BPP). The protein levels of Bax, Bak1, and caspase-3 were increased by 100 nM except for BPP and BPPH. The apoptosis rate was also increased since 100 nM (BPA, BPC) or at 10 μM (BPE, BPG, BPP, BPBP). BPA, BPE, BPC, BPG, and BPAP at 10 μM and BPAP at 100 nM also significantly increased Ca <sup>2+</sup> levels in IMR-32 cells. <i>Treatment for 3 h:</i> BPA, BPE, BPC, BPAP, and BPP at 10 μM increased intracellular Ca <sup>2+</sup> levels in IMR-32 cells, suggesting an activation of GPER. This effect was attenuated by GPER antagonist (G15) treatment (except for BPP). The ROS levels induced by BPC and BPAP were also reduced by G15. Molecular dynamics simulation suggests that BPA, BPE, BPC, and BPAP may activate GPER.	Wang L et al. (2023)
TCBPA, TBBPA, TBBPS, BDE-47, and BDE-209	TCBPA, TBBPA, TBBPS, BDE-47, as well as mix of 5 compounds: 0.01, 0.1, 1, and 5 μM BDE-209 doses: 10, 100, and 1000 nM	Human embryonic stem cell (HESC)	Deregulation of transcription factors determinant for neuronal development like ZIC1, ZIC3, HES3, IGFBP3 as well as DLX5 by the five targeted chemicals. All the 5 chemicals targeted dysregulated genes (e.g. CNTN2, SLIT1, LRRC4C, RELN, CBLN1, CHRN4, and GDF7) involved in axon growth/guidance and neuron transmission-related processes. AhR and WNT signaling pathways were impacted by all compounds. All five flame retardants significantly up-regulated CYP1A1 gene expression, suggesting that all of them could behave as agonists for the AhR signaling pathway.	Liang S et al. (2019)

Ach: acetylcholine; AMPAR:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; AhR: aryl hydrocarbon receptor; Bak1: BCL2 antagonist/killer 1; Bax: Bcl-2-associated X; BFR: brominated flame retardant; CBLN1: cerebellin 1 precursor; CGC: cerebellar granule cells; CHRN4: cholinergic receptor nicotinic beta 4 subunit; CNTN2: contactin 2; DLX5: distal-less homeobox 5; GDF7: growth differentiation factor 7; GPER: G protein-coupled estrogen receptor; HES3: Hes family BHLH transcription factor 3; HFR: halogenated flame retardant; hESCs: human embryonic stem cells; IGFBP3: insulin-like growth factor binding protein-3; LOEC: the lowest observed effect concentration; LRRC4C: leucine rich repeat containing 4C; NLRP3: NOD-, LRR-, and pyrin domain-containing protein 3; NMDAR: N-methyl-D-aspartate receptor; RELN: Reelin; ROS: reactive oxygen species; SLIT1: slit guidance ligand 1; ZIC: zinc finger protein.

The full version including also data on TBBPA is available in [Table A5](#).

is reasonable to hypothesize an immune adverse action of both bisphenols. BPE short-term exposure (24 h) was also able to induce oxidative stress, increasing the level of ROS on neuroblast cells at the concentration of 1 nM, 100 nM, and 10 μM (Wang L et al. 2023). Also in this case, the existing connection between oxidative stress and inflammation,

renders presumably an effect of BPZ and BPE on immunity (Lauridsen 2019).

There are relatively more data available for halogenated bisphenols such as TBBPA. This bisphenol was able to reduce tumor necrosis factor alpha (TNF- $\alpha$ ) levels in both peripheral blood mononuclear cells (PBMCs) and purified natural killer (NK) cells starting at the lowest tested concentration of 0.05–5  $\mu$ M (Yasmin and Whalen 2018). Similar effects were also observed on interferon gamma (IFN- $\gamma$ ) on human PBMC, monocyte-depleted PBMCs and purified NK cells, following 24 h, 48 h, or 6 days of exposure at the same concentrations active on TNF- $\alpha$  (Almughamsi and Whalen 2016). Instead, the secretion of interleukin (IL)-1 $\beta$  was increased in the same cells by the same concentrations of TBBPA 10 and 20  $\mu$ M (Anisuzzaman and Whalen 2016). In trophoblasts, TBBPA increased the release of IL-6 and IL-8 and reduced the release of transforming growth factor beta (TGF- $\beta$ ) (Park HR et al. 2014). NK lytic functionality was also reduced by TBBPA exposure starting from 0.5  $\mu$ M (Kibakaya et al. 2009). Furthermore, TBBPA has been linked to the downregulation of several genes linked to immune system at the dose of 250 mg/kg bw/day, suggesting an immunosuppressive effect *in vivo* (NTP 2013; Hall et al. 2017). In addition, TBBPA (10–100 nM) was able to affect gene transcription on mouse embryonic stem cells, at two main levels: neuronal differentiation and immune functionality, with a reduction of the membrane T cell receptor *Cd79a*, *Cd79b*, interleukin 7 receptor (*IL7r*), colony-stimulating factor 1 receptor (*Csf1r*), C-C motif chemokine receptor 2 (*Ccr2*), and an increase of the high affinity IgE receptor (*Fcer1g*) genes (Tribondeau et al. 2022).

Exposure of female mice to TCBPA (5 and 50 mg/kg bw/day) resulted in immune perturbations. In detail, it increased both pro- and anti-inflammatory cytokine levels in serum, namely IL-2, IL-4, IL-5, IL-10, IL-12, TNF- $\alpha$ , IFN- $\gamma$ , and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Wang Y et al. 2021). Exposure of mice for 14 days to the same TCBPA doses was able to decrease the percentage of T lymphocytes but to increase T regulatory cells, and the highest concentrations of TCBPA (100  $\mu$ g/mL) also included a reduced cell proliferation in mouse primary splenic lymphocytes exposed to TCBPA for 72 h (Wang Y et al. 2021).

#### 4.3. Concluding considerations

To date, there are limited available data on the immunotoxic potential of BPA alternatives. However, there is an indication of possible effects on the immune system for some of them, affecting both innate and acquired immunity, that could in turn contribute to several immune-mediated conditions. Additional studies are necessary to identify the most suitable BPA substitutes, also taking into account the metabolic competence of the *in vitro* system used.

### 5. Developmental neurotoxicity of BPA alternatives

#### 5.1. General considerations

Neural effects have contributed to the classification of BPA as a SVHC (ECHA 2016, 2017). These effects included impaired

spatial and non-spatial memory and modifications in neural processes related to synaptic plasticity (Mhaouty-Kodja et al. 2018). Developmental exposure to other bisphenols such as BPS also induced behavioral effects in rodents (Beausoleil et al. 2022). Whether BPA alternatives such as BPZ, BPE, BPP, BPAP, BPS-MAE or TBBPA and TCBPA have adverse effects comparable to BPA is discussed below. The retrieved *in vivo* and *in vitro* data are presented in Table 4 and in Tables A4 and A5, respectively.

#### 5.2. Neurotoxic effects of BPA analogues and halogenated bisphenols

##### 5.2.1. BPA analogues

A recent study assessed the potential neurodevelopmental effects of BPAP (Wu X et al. 2023) but no data are currently available for BPZ, BPE, BPP, or BPS-MAE. Prenatal and postnatal exposure of mice to 0.4 mg/kg/d of BPAP reduced the novel object recognition behavior, sociability, and social novelty behavior in adult males and females (Table A5). In addition, BPAP-exposed animals exhibited increased anxiety state level in the open-field, elevated plus maze, and marble burying tests. These behavioral modifications were associated with changes in hippocampal gene expression. In particular, genes associated with neurodevelopment including neural cell adhesion molecule 2 (*Ncam2*) and interleukin 1 receptor accessory protein like 1 (*Il1rap1l*) were down-regulated. In contrast, microglial and neuroinflammatory markers were upregulated, suggesting microglial activation that was confirmed at the protein level by increased immunoreactivity of ionized calcium-binding adapter molecule 1 in the hippocampus of BPAP-exposed mice.

An *in vitro* study examined the impact of BPA and its analogues BPS, BPF, BPE, BPB, BPZ, and BPAF on cell differentiation processes (Table 4). As human embryonic stem cells (hESCs) and hESC-derived neural stem cells (NSCs) can be differentiated into neural epithelium and neuron-like cells, respectively, they have been used to investigate the effects of bisphenol exposure during differentiation on gene and protein expression levels as well as on cell morphology (Liang X et al. 2020). BPAF was the most cytotoxic analogue in both cell models (0 up to 300  $\mu$ M). BPS, BPF, BPA, and BPAF all triggered morphological changes, with a significant reduction in neurite total length from the lowest dose tested (1 nM), while a slight decrease was shown for BPE, BPB, or BPZ at the highest dose tested (100 nM). When cells were exposed at concentrations that mimic a human exposure scenario (1–10 nM), no change was observed in the levels of expression of genes and proteins involved in the process of neural epithelium from hESC or specification of neuron-like cells from NSCs (Liang X et al. 2020).

A more recent study evidenced the potential of BPA and several of its alternatives, including BPP and BPE, to induce oxidative stress and apoptotic processes in a neuroblastoma cell line (Wang L et al. 2023). This effect involved mechanisms mediated by GPER, recognized as a major mediator of non-genomic estrogen signaling pathways (Alexander et al. 2017). Unlike the effects of bisphenols mediated by the



nuclear ERs, these effects occurred at relatively low concentrations, within the nM range for increased ROS levels with BPA and BPE. Although it increased caspase 3 expression by a factor of two, BPAP appeared to have the weakest effect on ROS expression and did not show any effect on apoptosis rate. The effect of BPA, BPE, and BPAP but not those of BPP on calcium mobilization and ROS formation were reversed by the GPER antagonist G15 highlighting the endocrine mediation of these adverse effects of bisphenols on this neural cell line (Wang L et al. 2023).

### 5.2.2. Halogenated bisphenols

Concerning the halogenated bisphenols, given that no *in vivo* data were available on the neurodevelopmental effects of TCBPA, the review focused on the effects of TBBPA. Hence, we analyzed the nine *in vivo* studies addressing the effects of prenatal/postnatal and prepubertal/pubertal exposure to TBBPA, as presented in Table A4.

In eight studies, the effects of prenatal/postnatal exposure to TBBPA were demonstrated at the doses indicated in Table A4. These included effects on the auditory response in rat, with suggested cochlear effect in females and a more apparent neural effect in males (Lilienthal et al. 2008). The benchmark doses of auditory effects were comparable to those reducing TH levels, and lower than those reducing maternal body weight. Perinatal exposure of rats to TBBPA at 0.1 mg/kg/d increased wheel activity in females, and elevated anxiety-state level in males after continuous exposure to TBBPA at 25 and 250 mg/kg/d (Rock et al. 2019). Postnatal exposure to a TBBPA bis(2-hydroxyethyl ether) (TBBPA-BHEE), a representative TBBPA derivative, also triggered motor coordination and activity deficits in prepubertal male rats at the two doses used of 0.086 and 0.86 mg/kg (Liu QS et al. 2018). Acute postnatal exposure of male mice to TBBPA increased activity in the open-field test at 5 mg/kg, freezing in the contextual fear conditioning paradigm at 0.1 and 5 mg/kg, and spontaneous alternation in the Y maze at 0.1 mg/kg (Nakajima et al. 2009). Deficits in memory retention using the passive avoidance test (Wang J and Dai 2022) and in spatial memory using the Morris water maze (Kim AH et al. 2017) have been also described following prepubertal/pubertal exposure of male mice to TBBPA at doses above 50 mg/kg/d. Finally, a reduction in social interaction was induced in adult male rats exposed prenatally and postnatally to TBBPA at 0.2 mg/kg/d (Kim B et al. 2015).

At the neuroanatomical level, changes have been reported in the number of hippocampal interneurons in the developing brain (Saegusa et al. 2012). Alterations in gene expression profile analyzed by microarray in rats exposed to TBBPA-BHEE (Liu QS et al. 2018), as well as in hippocampal neurogenesis (Kim AH et al. 2017), oxidative stress, apoptosis markers, brain-derived neurotrophic factor (BDNF), and neurotransmitter levels in mice have also been described (Wang J and Dai 2022). It has been suggested that TBBPA-induced neurodevelopmental effects involved direct effects on the brain (Saegusa et al. 2012) and indirect actions through modifications in TH (Lilienthal et al. 2008). When male mice were exposed to a single postnatal TBBPA dose of

11.5 mg/kg, no changes were observed in the levels of proteins related to hippocampal neuroplasticity (Viberg and Eriksson 2011).

In one study assessing the effects of developmental exposure to TBBPA on the F2 generation of rats born from F1 parents exposed prenatally to 10–1000 mg/kg/d of TBBPA, Cope et al. (2015) reported no detectable neurodevelopmental or neurobehavioral deficits.

In addition to the *in vivo* studies, there are *in vitro* studies mostly focused on the neurotoxic effects of TBBPA (see Table A5). Exposure to TBBPA triggered oxidative stress, intracellular  $\text{Ca}^{2+}$  imbalance, elevation of extracellular glutamate and death in primary culture of cerebellar granule cells (CGCs) prepared from seven-day old Wistar rats (Reistad et al. 2007; Zieminska et al. 2017; Bowen et al. 2020). The increase in intracellular  $[\text{Ca}^{2+}]$ , oxidative stress, and mitochondria depolarization was dose-dependent from 10 to 25  $\mu\text{M}$  (Al-Mousa and Michelangeli 2012; Zieminska et al. 2016; Diamandakis et al. 2019). The authors hypothesized that microsomal Ca-ATPase inhibition could play a crucial role in  $\text{Ca}^{2+}$  homeostasis, which in turn induces mitochondrial dysfunction (Al-Mousa and Michelangeli 2012). In similar conditions of treatment, TBBPA also impaired the homeostasis of  $\text{Zn}^{2+}$  in neuronal cells (Zieminska et al. 2017; Bowen et al. 2020). Acute exposure of CGC to TBBPA (10 or 25  $\mu\text{M}$ ) triggered a steady decline in cell viability (from 3 h up to 24 h) associated with an increasing number of neurons with irregularities in their nuclei. At the highest dose, TBBPA altered gene expression involved in the major signaling pathways that lead to neuronal death. A decrease in the expression of autophagy and anti-apoptotic genes was observed in the first 3 h of exposure, followed, in a second step, by the activation of apoptosis-associated genes from 6 to 24 h post exposure. Expression of programmed necrosis-associated genes was not altered regardless of the exposure time (Lenart et al. 2017). Similar results were obtained on primary hippocampal neuronal cultures prepared from Swiss mouse embryo at gestational days 17–18. Exposure to TBBPA at doses ranging from 100 nM to 100  $\mu\text{M}$  similarly led to apoptotic cell death, although in these cells it was accompanied by a certain level of necrotic cell death (Szychowski and Wójtowicz 2016). The ability of TBBPA to induce cell death, at doses ranging from 3 to 10  $\mu\text{M}$ , at least partially by apoptosis, through the activation of caspase has also been described in SH-SY5Y human neuroblastoma cells at low  $\mu\text{M}$  levels (Al-Mousa and Michelangeli 2012). The authors pointed out a clear relationship between the alteration of  $\text{Ca}^{2+}$  homeostasis within neuronal cells and the initiation of neuronal cell degeneration and death. Furthermore, the damage done by 12 h of TBBPA exposure increased secretion of  $\beta$ -amyloid-42 levels, a key molecular factor in neurotoxicity (Al-Mousa and Michelangeli 2012) and expression of the presynaptic synaptosomal-associated protein, 25 kDa (SNAP-25) that controls neurotransmitter release and plasma membrane growth (Zieminska et al. 2016). These results suggest that SNAP-25 might be a target leading to cognitive ability impairments. Ototoxicity was induced by exceptionally high TBBPA levels (125  $\mu\text{g}/\text{mL}$ ) in HEI-OC1 (Park C et al. 2016). This observation agrees with the significant increase in the percentage of apoptotic cells in Corti organ explants from postnatal day 2 rats exposed to TBBPA for 18–



**Table 5.** Summary of studies addressing the prioritized BPA alternatives' genotoxicity.

Substance	Cell model	Assay	Exposure conditions	Effect	Reference
BPAP	HepG2 3D spheroids	Comet assay	0.01, 0.1, 1, 10, and 40 $\mu$ M, for 24 h 0.01, 0.1, 1, and 10 $\mu$ M, for 96 h	Positive at the highest dose Positive at $\geq 0.1 \mu$ M	Sendra et al. (2023)
		$\gamma$ H2AX	0.01, 0.1, 1, 10, and 40 $\mu$ M, for 24 h 0.01, 0.1, 1, and 10 $\mu$ M, for 96 h	Negative Positive	
	RAD54 <sup>-/-</sup> mutant chicken DT40 cells	$\gamma$ H2AX	31.3 $\mu$ M, for 1 h	Negative	Lee S et al. (2013)
		Chromosomal aberration	7.8, 15.6, and 31.3 $\mu$ M, for 48 h	Negative	
BPP	RAD54 <sup>-/-</sup> mutant chicken DT40 cells	$\gamma$ H2AX	12.5 $\mu$ M, for 1 h	Positive	Lee S et al. (2013)
		Chromosomal aberration	3.13, 6.25, and 12.5 $\mu$ M, for 48 h	Positive at the highest dose	
BPZ	HepG2 cells	Comet assay	0.1, 1, and 10 $\mu$ M, for 4 and 24 h	Positive at $\geq 1 \mu$ M, for 4 h No cytotoxicity at 12.5, 25, 50, and 100 $\mu$ M, for 24 h	Fic et al. (2013)
	<i>S. Typhimurium</i> strains TA98, TA100	Ames test	4, 20, 100, and 500 $\mu$ g/plate $\pm$ S9	Negative	

$\gamma$ H2AX: histone family member X; HepG2 cells: human hepatoma-derived cell line; RAD54<sup>-/-</sup>: defective in *RAD54* gene; *S. typhimurium*: *Salmonella typhimurium*.

24 h (Park C et al. 2016). However, the levels of exposure used to observe these neuro- and ototoxic effects are far from being representative of human exposure levels, which would be more in the  $\mu$ M or even nM range (Liang X et al. 2020). The capacity of TBBPA to induce mitochondrial bioenergetic impairments was also investigated in murine BV-2 microglial cells. BV-2 cells remained viable ( $\geq 80\%$  of viability) following exposure to TBBPA concentrations ranging from 10 to 40  $\mu$ M (Bowen et al. 2020). However, although weak changes in pro-inflammatory responses were observed, there were significant alterations in mitochondrial energy production, including ATP-linked respiration, basal, and compensatory glycolysis. The phagocytotic potential was also decreased (Bowen et al. 2020).

Only one *in vitro* study investigated TCBPA-induced effects (Liang S et al. 2019) (Table 4). The neurotoxic effects of TBBPA alone or in combination with other flame retardants (BDE-47, BDE-209, TBBPS, and TCBPA) were investigated on induced neural ectoderm from hESCs (Liang S et al. 2019). RNA-seq analyses highlighted that all the halogenated flame retardants (HFRs) targeted triggered dysregulation in the expression of genes involved in (i) transcription factors determinant for neural development, (ii) axon growth/guidance, and (iii) neuron transmission processes. Assays conducted on HFR mixture did not show clear synergic effects (Liang S et al. 2019). Based on the obtained results, Liang S et al. (2019) concluded that, from a neurotoxic point of view, TCBPA should not be considered as safe alternative to TBBPA.

### 5.3. Concluding considerations

The SVHC classification of BPA supported the plausibility that the impaired learning and memory and underlying synaptic plasticity processes induced by this substance were essentially mediated through disruption of the estrogenic signaling pathway (Mhaouty-Kodja et al. 2018). Major endocrine

systems such as the steroid and thyroid pathways are well-known regulators of neural development and cognitive function (Hamson et al. 2016; Mughal et al. 2018). Alterations of these pathways whether at the maternal or the fetal levels during critical windows of neurodevelopment might result in long-term effects. To our knowledge, there are no *in vivo* data linking neurodevelopmental effects of BPA alternatives to an endocrine mode of action. The available *in vitro* data presented in Section 3 together with the study of Wang J and Dai (2022) suggest the ability of some bisphenol alternatives to bind and activate receptors for sex steroid and THs, but more studies are still required to confirm this hypothesis.

Overall, the scarce data reported for BPA alternatives (one *in vivo* study for BPAP and two *in vitro* studies for several BPA analogues) together with the available data on TBBPA suggest that exposure to BPA alternatives might induce potential neurodevelopmental effects. However, additional studies are required to address these aspects. In addition, although DNT with an endocrine component was one main endpoint that led to the classification of BPA as a SVHC at the EU level, data gaps prevent a proper evaluation of the BPA alternatives/analogues effects on DNT via endocrine-mediated mechanisms.

## 6. Genotoxicity and carcinogenicity of BPA alternatives

### 6.1. General considerations

The characterization of the carcinogenic potential of BPA alternatives is central for their hazard and risk assessment. For the classification of chemicals' carcinogenicity, the International Agency for Research on Cancer (IARC) considers all relevant and reliable data contributing to the knowledge on carcinogenicity in experimental animals, and mechanistic evidence from *in vitro* and *in vivo* models (IARC 2019).

Regarding the mechanistic evidence, Smith et al. (2016) proposed the application of the 10 key characteristics of human carcinogens (e.g. genotoxicity, induction of cell proliferation, immunosuppression or epigenetic effects, among others) to systematically identify and organize mechanistic information about a given agent. Through this approach, Ricker et al. (2024) concluded that BPA may act by multiple and interconnected mechanisms. Indeed, exposure to BPA has been associated with breast, ovarian, prostate, cervical, and lung cancer development, possibly due to its ED activity (Seachrist et al. 2016; Almeida et al. 2018; Khan et al. 2021; Winz and Suh 2021). Several rodent studies showed that BPA influences the action of estrogen and progestin agonists through interactions with ERs, GPER, and PR, leading to mammary tissue hyperplasia and cancer development (Vandenberg et al. 2008; Perrot-Applanat et al. 2018), which contributed to the BPA classification as a SVHC (ECHA 2017). The assessment of BPA genotoxicity through *in vitro* testing showed no mutagenic or clastogenic potential in some studies (Ivett et al. 1989; Schweikl et al. 1998; Tsutsui et al. 1998; Audebert et al. 2011; Fic et al. 2013) while others evidenced its ability to produce DNA and chromosomal damage, particularly in human liver-derived cell lines (Fic et al. 2013; Sendra et al. 2023; Yu H and Liu 2023) and in ER-positive MCF-7 cells (Iso et al. 2006). Overall, based on the available data, EFSA concluded that it is unlikely to very unlikely that BPA presents a genotoxic hazard by a direct mechanism of action (EFSA 2023).

## 6.2. Information on the genotoxicity and carcinogenicity of BPA alternatives

Recent studies have supported the effects of data-rich BPA alternatives (e.g. BPF and BPS) and other less studied alternatives, including TCBPA, on cell proliferation and cell migration through an ER-dependent pathway (Lei et al. 2018; Awada et al. 2019; Huang et al. 2019; Jung et al. 2019; Lei, Tang, et al. 2021; Lei, Xu, et al. 2021; Yu M et al. 2023). This suggests that those chemicals cannot be considered *a priori* safe alternatives for BPA replacement (Edaes and de Souza 2022).

Regarding the genotoxicity of the prioritized BPA alternatives, Table 5 summarizes the main knowledge available in the literature. A recent *in vitro* study showed that BPAP (0.01–10  $\mu\text{M}$ , 96 h exposure) induces the formation of DNA and chromosome breaks (using the comet and the  $\gamma\text{H2AX}$  assays, respectively) in human liver-derived HepG2 3D spheroids (Sendra et al. 2023). However, no clastogenicity had been previously observed in  $\text{RAD54}^{-/-}$  mutant chicken DT40 cells (Lee S et al. 2013). BPP showed genotoxic potential by inducing chromosomal aberrations (12.5  $\mu\text{M}$ , 1 h) and  $\gamma\text{H2AX}$  foci formation (12.5  $\mu\text{M}$ , 48 h) in the referred DT40 cells (Lee S et al. 2013). BPZ was able to induce DNA strand breaks (1 and 10  $\mu\text{M}$ , 4 h exposure) in HepG2 cells. BPZ, similarly to other BPA alternatives (BPF, BPAF, and BPS) was not mutagenic in the Ames test, with and without exogenous S9 metabolic fraction (Fic et al. 2013). For the remaining prioritized BPA alternatives, i.e. BPE, BPS-MAE, and TCBPA, no published data on their potential genotoxicity were found, which does not exclude the existence of datasets, e.g. as parts of

chemicals' request for authorization by competent authorities.

On the other hand, there is information on other BPA alternatives' genotoxicity and the data available, particularly, regarding BPS, BPF, and BPAF, are also briefly presented due to their relevance to understand possible mechanisms of action of the prioritized analogues (see Table A6). BPF was shown to be genotoxic in HepG2 cells (Fic et al. 2013; Lee S et al. 2013; Hercog et al. 2019; Yu H and Liu 2023). Likewise, BPS was mostly genotoxic by inducing DNA breaks and/or chromosomal alterations in HepG2 (Fic et al. 2013; Sendra et al. 2023; Yu H and Liu 2023), human prostatic (Kose et al. 2020), and bronchial BEAS-2B cells (George and Rupasinghe 2018), and also in a humanized peripheral blood mononuclear cell (Hu-PBMC) model (Mokra et al. 2017). Several studies have also evidenced BPAF genotoxicity, especially showing a clastogenic effect (Lee S et al. 2013; Mokra et al. 2017; Hercog et al. 2019; Sendra et al. 2023). Mokra et al. (2017) observed that BPAF and BPS were the substances bearing the highest and the lowest genotoxic potential in Hu-PBMCs, respectively. BPAF was the most potent inducer of oxidative DNA damage, whereas BPS induced the lowest level, suggesting that oxidative damage was implicated in the observed genotoxicity (Mokra et al. 2018). The authors associated the ability of bisphenols to induce oxidative DNA damage to their possible intracellular biotransformation into highly reactive hydroxylated metabolites, which could be further transformed into quinones via CYP 2E1, existent in mammalian lymphocytes (Mokra et al. 2018). The importance of the *in vitro* cell systems' metabolic competence to reliably assess the genotoxicity of BPA alternatives was demonstrated by Yu H et al. (2020). Using Chinese hamster lung-derived cell lines expressing various human CYP enzymes and a human (metabolism-proficient) hepatoma C3A cell line, the authors showed that BPF and BPS (40, 80, and 160  $\mu\text{M}$ , for 9 h followed by a 15 h of recovery), like BPA, were clastogenic (Yu H et al. 2020). Consistent with those observations, Hercog et al. (2019) reported that BPF, BPS, and BPAF up-regulated the expression of CYP1A1 in HepG2 cells. Moreover, they observed a negative clastogenicity modulation by phase II enzymes (SULTs and UGTs) (Yu H et al. 2020) suggesting that inactive metabolites are formed. Audebert et al. (2011) had previously shown that BPF was extensively metabolized by HepG2 and LS174T intestine cells and produced a genotoxic effect.

Although some studies have suggested that bisphenols increase intracellular ROS through an estrogen-mediated pathway (Lei et al. 2018), ER-independent oxidative effects and chromosomal alterations have also been observed (Špačková et al. 2020). It is therefore plausible that the genotoxicity of these substances may be mediated by (ER-dependent or -independent) oxidative stress (Ribeiro et al. 2017). Epigenetic effects such as DNA methylation, histone modification, and the expression of noncoding RNAs are some of the epigenetic targets of BPA and its alternatives (reviewed by Almeida et al. 2018) that might explain an estrogen-independent genotoxic and carcinogenic mode of action. However, the link between DNA damage caused by bisphenols exposure and epigenetic effects remains unclear.

### 6.3. Concluding considerations

Overall, the knowledge about potential genotoxic effects of the selected BPA alternatives is very limited. Although some evidence exists for the *in vitro* genotoxicity of BPAP, BPP, and BPZ, no published data were found for the other prioritized chemicals. Given the ability of other more data-rich BPA alternatives (BPS, BPF, and BPAF) to induce genotoxic effects, possibly mediated by oxidative stress after undergoing phase I metabolism, there is an urgent need for expanding the knowledge on the BPA alternatives in focus. Low-concentration ranges relevant for human exposure should be tested under standardized conditions, using metabolism-proficient cell lines or external metabolic systems to account for the potential effect of metabolites. In addition, considering the key characteristics of carcinogens, other features, such as the ability of altering cells proliferation, inducing oxidative stress, or cell transformation, among others, should be investigated.

## 7. Conclusions, knowledge gaps, and future directions

Although manufacturers have already started using alternative chemicals to BPA for many applications, there are a number of features which should be considered to avoid regrettable substitutions of BPA in particular, and of any chemical of concern, in general. Among those features, which include performance (similar functional properties), biodegradability, cost-effectiveness, transparency, and innovation, those related to safety and regulatory compliance are of utmost importance. While more data on potential adverse effects of already marketed BPA alternatives are needed, those under development should undergo rigorous safety assessment. Ideally, they should have a proven track record of safety through comprehensive testing, possibly using NAMs. This means that BPA should be replaced by nontoxic chemicals, both in their raw form and when used in consumer products. This includes ensuring that they do not leach harmful chemicals into food, beverages, or the environment, even under conditions such as heating or acidic environments. Despite the data already produced on their biological activity, most alternatives to BPA still present a major gap of knowledge regarding their metabolism and kinetics, as well as effects on endocrine and immune systems, neurodevelopment, genotoxic and carcinogenic properties, as detailed in the concluding considerations at the end of each section or subsection.

The generation of kinetic data has been identified as one of the key research priorities needed to improve mechanistic understanding of chemical-induced toxicity (European Commission 2012; EFSA 2014; Escher et al. 2022), and the knowledge on BPA kinetic behavior has been crucial to decrease the uncertainties related to its risk assessment based on toxicity data produced in animal models (EFSA 2010, 2015, 2023). On the other hand, when NAMs are used and integrated in the NGRA, kinetics data are essential as PBK input and for developing a robust QIVIVE. Despite these considerations, kinetic data are available for a few analogues, such as BPS and BPF, while scant, fragmentary or no

information could be found for the alternatives selected within the PARC project.

The review highlighted that the reported *in vitro* toxicity data are generally obtained with cell lines endowed with no, scant, or unknown metabolic competence, thus assessing the activity of the parent compound. This may represent a substantial source of overestimation or underestimation of the overall hazard, depending on whether metabolism generates inactive or bioactive metabolites, respectively. Therefore, the use of metabolism-proficient *in vitro* cell models becomes crucial for accurate dose–response identification with these models. In addition, as previously discussed, the concentrations tested *in vitro* are frequently much higher with respect to the possible human exposure levels indicated by biomonitoring studies (in the nM to low  $\mu$ M range). The extrapolation to low dose can be another relevant source of uncertainty.

In summary, it will be important to study the potential mode(s) of action of BPA alternatives, in order to establish their ED potency relative to that of BPA, and to obtain comparable data using the same cellular systems and harmonized methodologies. In addition, data still need to be generated concerning their DNT or immunotoxicity, considering that the neural effects were among the endpoints leading to the classification of BPA as a SVHC at the EU level (ECHA 2017), and immunotoxic effects were used for the health-based reference value in the most recent EFSA opinion on BPA risk assessment (EFSA 2023). Furthermore, despite the scarcity of data, there are studies showing that some BPA alternatives own key characteristics of carcinogens, including the potential to produce genotoxicity, epigenome alterations, and cell proliferation, especially in estrogen-insensitive tissues, such as the liver. Therefore, there is a clear need to also produce data on these endpoints and underlying mechanisms, in order to clarify whether the BPA alternatives in use might contribute to the development of cancer mediated (or not) by a genotoxic mechanism.

The data available and reviewed here also evidenced that, despite a lot of similarities with BPA, the different alternatives also show a number of differences in their kinetics and induced effects. A good example is given by the wide spectrum of estrogenic activities, with TBBPA being almost inactive, while others display a different level of activity toward the various ERs with no information available on the biotransformation products. The same considerations can be applied to the other receptor binding activities and, as a consequence, the potentially mediated toxic effects. This implies that in most cases the read across principle with data on BPA or on other data-rich BPA analogues (i.e. BPS or BPF) should be used with caution, possibly based on robust bridging studies obtained with the different BPA alternatives.

The reported data originated from an extensive literature search (although the papers were not retrieved following the strict principles of a systematic review) carried out by experts in the field of each endpoint, in order to obtain a level of detail appropriate for the aim of the PARC project and the target audience. In addition, the main focus was on BPA alternatives relevant for the European Regulatory Agencies, as the major stakeholders within the frame of the PARC project.

Some of the conclusions drawn in the present review are shared by another recent review also related to PARC activities that summarized, from the environmental perspective, the gaps and needs on BPA alternatives (Adamovsky et al. 2024). In agreement with the present analysis, nearly 70% of studies using vertebrate models focused on BPS, BPF, BPAF, and TBBPA. Adamovsky et al. (2024) stressed the importance of conducting more comprehensive assessments that go beyond the traditional reproductive studies and focus on overlooked relevant endpoints, such as the immune system. Future research should also consider mixture effects, realistic environmental concentrations, and the long-term consequences on biota and ecosystems.

Overall, from the present review we conclude that there is an urgent need to (i) determine the mechanism(s) through which BPA alternatives (and/or their metabolites) act, (ii) develop relevant *in vitro* tools that allow assessing simultaneously ED and other adverse outcomes, and (iii) search for molecular biomarkers that allow predicting immunotoxicity, endocrine disruption, non-genotoxic carcinogenesis, and/or DNT of novel BPA alternatives, before they come into widespread use. A step forward should also include the evaluation of sex-specific effects due to sexual dimorphism of many of the endpoints herein discussed. Moreover, the future *in vitro* studies should (i) test concentration ranges close to the actual human exposure, (ii) include, whenever possible, biokinetic measurements showing intracellular uptake and distribution, and (iii) have the potential to study the effects of metabolites by using metabolism-competent cells.

Within PARC, the majority of identified gaps will be addressed and studying the key steps of BPA alternatives' mode(s) of action will be a crucial process underlying a tight synergism with other internal projects to improve/build AOPs, including also kinetics data. This is relevant for all endpoints selected, crucial to classify or not the selected substances as EDCs, as well as to determine if they are safe alternatives to BPA by carrying out an adequate quantitative risk assessment.

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## Appendix

Table A1. Summary of the *in vitro* assays assessing effects on nuclear and membrane receptors exerted by BPA analogues.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
BPAP	ER $\alpha$	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 255 $\pm$ 41.0	Liu X et al. (2019)
		Competitive fluorescence polarization binding assay		IC <sub>50</sub> 2.6 $\mu$ M	Keminer et al. (2020)
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 258 $\pm$ 23.6	Liu X et al. (2021)
		MVLN cells, ER $\alpha$ binding affinity	1 nM to 100 $\mu$ M	No effect	Lin et al. (2021)
		MCF7 – luciferase reporter gene assay	10 pM to 100 $\mu$ M	Agonist; AC <sub>50</sub> 1 $\mu$ M	Mesnage et al. (2017)
		HELN-ER $\alpha$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.761 $\pm$ 0.151; max efficacy 40%	Grimaldi et al. (2019)
		CHO-K1 – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 1.9 $\mu$ M	Kojima et al. (2019)
		HepG2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)
		Yeast two-hybrid assay	10 pM to 10 $\mu$ M	Not significant	van Leeuwen et al. (2019)
		MVLN – luciferase reporter gene assay		Antagonist; logRIC <sub>20</sub> –7.61 (M)	Chen Q et al. (2020)
		HeLa – luciferase reporter gene assay	10 pM to 10 $\mu$ M	Agonist; EC <sub>50</sub> (nM) 351 $\pm$ 33	Liu X et al. (2021)
		OECD TG 455 – STTA assay on hER $\alpha$ -HeLa-9903 cells	10 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 2.00 $\pm$ 1.29; max effect 66.63% $\pm$ 3.90	Durcik, Hiti, et al. (2022)
		MCF7 – proliferative potential (E-screen assay)	10 pM to 100 $\mu$ M	Induction; AC <sub>50</sub> 0.39 $\mu$ M	Mesnage et al. (2017)
	ER $\beta$	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 62.1 $\pm$ 9.27	Liu X et al. (2019)
		Competitive fluorescence polarization binding assay		No effect	Keminer et al. (2020)
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 68.0 $\pm$ 9.57	Liu X et al. (2021)
		HELN-ER $\beta$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.61 $\pm$ 0.28; max efficacy 40%	Grimaldi et al. (2019)
		CHO-K1 – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 0.17 $\mu$ M	Kojima et al. (2019)
		HepG2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)
		HeLa – luciferase reporter gene assay	10 pM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> (nM) 595 $\pm$ 56.0	Liu X et al. (2021)
	ERR $\gamma$	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 120 $\pm$ 4.20	Liu X et al. (2019)
		HG5LN-ERR $\gamma$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)
	AR	Competitive receptor binding affinity assay		IC <sub>50</sub> > 10 $\mu$ M	Liu X et al. (2019)
		Competitive fluorescence polarization binding assay		IC <sub>50</sub> 5.4 $\mu$ M	Keminer et al. (2020)
		HELN-AR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> ( $\mu$ M) 15.9 $\pm$ 3.3	Grimaldi et al. (2019)
		CHO-K1 – luciferase reporter gene assay	100 nM to 10 $\mu$ M	Antagonist; RIC <sub>50</sub> 8.5 $\mu$ M	Kojima et al. (2019)
		HepG2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)
Yeast two-hybrid assay		10 pM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> 40 $\mu$ M	van Leeuwen et al. (2019)	
MDA-kb2 – luciferase reporter gene assay		0.1 pM to 10 $\mu$ M	Antagonist	Ma M et al. (2022)	
22Rv1 – protein expression/localization		10 nM to 50 $\mu$ M	Antagonist; IC <sub>50</sub> 16 $\mu$ M	Stossi et al. (2016)	
PR	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 330 $\pm$ 21.9	Liu X et al. (2019)	
	HELN-PR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> ( $\mu$ M) 23.1 $\pm$ 9.4	Grimaldi et al. (2019)	
GR	MCF7 – gene expression	1 $\mu$ M	Induction	Pelch KE et al. (2019)	
	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 30.1 $\pm$ 0.923	Liu X et al. (2019)	
		HMLN-GR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)

(continued)

Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference	
BPE	PPAR $\gamma$	CHO-K1 – Luciferase reporter gene assay	100 nM to 10 $\mu$ M	Antagonist; RIC <sub>50</sub> 8.1 $\mu$ M	Kojima et al. (2019)	
		MDA-kb2 – Luciferase reporter gene assay		Agonist; logLOEC –6.80 (M)	Chen Q et al. (2020)	
		CHO-K1 – Luciferase reporter gene assay		No effect	Chen Q et al. (2020)	
		MDA-kb2 – Luciferase reporter gene assay	0.1 pM to 10 $\mu$ M	Agonist/antagonist	Ma M et al. (2022)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> > 10 $\mu$ M	Liu X et al. (2019)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 1060 $\pm$ 93.2	Liu X et al. (2019)	
	PXR	HG5LN-PXR – Luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 65.9 $\pm$ 15; max efficacy 30%	Grimaldi et al. (2019)	
		COS-7 – Luciferase reporter gene assay	100 nM to 10 $\mu$ M	Agonist; REC <sub>20</sub> 9.7 $\mu$ M	Kojima et al. (2019)	
	ER $\alpha$	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) = 5300 $\pm$ 318	Liu X et al. (2019)	
		BG1Luc4E2 – reporter gene assay (OECD 457)	100 nM to 100 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.47 $\pm$ 0.08; max efficacy 386 $\pm$ 133	Rosenmai et al. (2014)	
		Bioluminescence yeast estrogen screen assay		Agonist; EC <sub>50</sub> 7.43 $\mu$ M	Ruan et al. (2015)	
		Yeast two-hybrid assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> 0.102 $\mu$ M	Conroy-Ben et al. (2018)	
		HELN-ER $\alpha$ – Luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.843 $\pm$ 113; max efficacy 72%	Grimaldi et al. (2019)	
		CHO-K1 – Luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 0.28 $\mu$ M	Kojima et al. (2019)	
		HepG2 – Luciferase reporter gene assay	3 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 1.4 $\mu$ M; max efficacy 88.4%	Pelch KE et al. (2019)	
		Yeast two-hybrid assay	10 pM to 10 $\mu$ M	Agonist; EC <sub>50</sub> 7 $\mu$ M	van Leeuwen et al. (2019)	
		MVLN – Luciferase reporter gene assay		Agonist; logREC <sub>20</sub> –6.16 (M)	Chen Q et al. (2020)	
		OECD TG 455 – STTA assay on hER $\alpha$ -HeLa-9903 cells	10 nM to 25 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 2.26 $\pm$ 0.87; max effect 127.47% $\pm$ 34.68	Durcik, Hiti, et al. (2022)	
		ER $\beta$	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) = 3900 $\pm$ 82.7	Liu X et al. (2019)
			HELN-ER $\beta$ – Luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.69 $\pm$ 0.088; max efficacy 87%	Grimaldi et al. (2019)
			CHO-K1 – Luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 94 nM	Kojima et al. (2019)
HepG2 – Luciferase reporter gene assay			3 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 4.6 $\mu$ M; max efficacy 118.7%	Pelch KE et al. (2019)	
	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) = 4.78 $\pm$ 0.608	Liu X et al. (2019)		
ERR $\gamma$	HG5LN-ERR $\gamma$ – Luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.69 $\pm$ 0.28; max efficacy 131%	Grimaldi et al. (2019)		
	Competitive receptor binding affinity assay		IC <sub>50</sub> > 10 $\mu$ M	Liu X et al. (2019)		
AR	CHO – reporter gene assay	0.4–100 $\mu$ M	Antagonist; EC <sub>50</sub> ( $\mu$ M) 1.9 $\pm$ 0.9; max efficacy 72% $\pm$ 11	Rosenmai et al. (2014)		
	Yeast two-hybrid assay	1 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> 6.1 $\mu$ M	Conroy-Ben et al. (2018)		
HELN-AR – Luciferase reporter gene assay		1 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> ( $\mu$ M) 1.46 $\pm$ 0.68	Grimaldi et al. (2019)		
	CHO-K1 – Luciferase reporter gene assay	100 nM to 10 $\mu$ M	Antagonist; RIC <sub>50</sub> 0.99 $\mu$ M	Kojima et al. (2019)		
	MDA-kb2 – Luciferase reporter gene assay	3 nM to 10 $\mu$ M	Antagonist; REC <sub>50</sub> 1 $\mu$ M; max efficacy 89.2%	Pelch KE et al. (2019)		
	Yeast two-hybrid assay	10 pM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> 10 $\mu$ M	van Leeuwen et al. (2019)		
	AR-CALUX	10 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> 1.1 $\mu$ M	Šauer et al. (2021)		
	MDA-kb2 – Luciferase reporter gene assay	0.1 pM to 10 $\mu$ M	Antagonist	Ma M et al. (2022)		
PR	Competitive receptor binding affinity assay		Inert	Liu X et al. (2019)		
	HELN-PR – Luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)		

(continued)

Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference	
	GR	PR-CALUX	10 nM to 10 μM	No effect	Šauer et al. (2021)	
		MCF7 – gene expression	1 μM	Induction	Pelch KE et al. (2019)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> > 10 μM	Liu X et al. (2019)	
		HMLN-GR – luciferase reporter gene assay	1 nM to 10 μM	No effect	Grimaldi et al. (2019)	
		CHO-K1 – luciferase reporter gene assay	100 nM to 10 μM	No effect	Kojima et al. (2019)	
		MDA-kb2 – luciferase reporter gene assay		No effect	Chen Q et al. (2020)	
		CHO-K1 – luciferase reporter gene assay		No effect	Chen Q et al. (2020)	
		MDA-kb2 – luciferase reporter gene assay	0.1 pM to 10 μM	Agonist	Ma M et al. (2022)	
		PPARγ	Competitive receptor binding affinity assay		Inert	Liu X et al. (2019)
			PXR	Competitive receptor binding affinity assay		IC <sub>50</sub> > 10 μM
HG5LN-PXR – luciferase reporter gene assay	1 nM to 10 μM			No effect	Grimaldi et al. (2019)	
COS-7 – luciferase reporter gene assay	100 nM to 10 μM			No effect	Kojima et al. (2019)	
BPP	ERα	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 152 ± 17.2	Liu X et al. (2019)	
		MVLN cells, ERα binding affinity	1 nM to 100 μM	Agonist; EC <sub>50</sub> 0.671 μM; max induction 35.4% (vs. E2)	Lin et al. (2021)	
		HELN-ERα – luciferase reporter gene assay	1 nM to 10 μM	Antagonist; IC <sub>50</sub> (μM) 1.91 ± 0.69	Grimaldi et al. (2019)	
		CHO-K1 – luciferase reporter gene assay	1 nM to 10 μM	Agonist; REC <sub>20</sub> 0.64 μM	Kojima et al. (2019)	
		HepG2 – luciferase reporter gene assay	3 nM to 10 μM	Not significant	Pelch KE et al. (2019)	
		Yeast two-hybrid assay	10 pM to 10 μM	Not significant	van Leeuwen et al. (2019)	
		MVLN – luciferase reporter gene assay		Antagonist; logRIC <sub>20</sub> -8.42 (M)	Chen Q et al. (2020)	
		ERβ	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 105 ± 10.8	Liu X et al. (2019)
			HELN-ERβ – luciferase reporter gene assay	1 nM to 10 μM	Antagonist; IC <sub>50</sub> (μM) 1.81 ± 0.52	Grimaldi et al. (2019)
			CHO-K1 – luciferase reporter gene assay	1 nM to 10 μM	No effect	Kojima et al. (2019)
ERRγ	HepG2 – luciferase reporter gene assay	3 nM to 10 μM	Antagonist; REC <sub>50</sub> 0.93 μM; max efficacy 84.1%	Pelch KE et al. (2019)		
	Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 320 ± 28.5	Liu X et al. (2019)		
	AR	HG5LN-ERRγ – luciferase reporter gene assay	1 nM to 10 μM	No effect	Grimaldi et al. (2019)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 409 ± 38.4	Liu X et al. (2019)	
	AR	HELN-AR – luciferase reporter gene assay	1 nM to 10 μM	Antagonist; IC <sub>50</sub> (μM) 10.8 ± 6.5	Grimaldi et al. (2019)	
		CHO-K1 – luciferase reporter gene assay	100 nM to 10 μM	Antagonist; RIC <sub>50</sub> 7.8 μM	Kojima et al. (2019)	
		MDA-kb2 – luciferase reporter gene assay	3 nM to 10 μM	Antagonist; REC <sub>50</sub> 6.5 μM; max efficacy 62.5%	Pelch KE et al. (2019)	
		Yeast two-hybrid assay	10 pM to 10 μM	Antagonist; IC <sub>50</sub> 2 μM	van Leeuwen et al. (2019)	
		AR-CALUX	10 nM to 10 μM	No effect	Šauer et al. (2021)	
	PR	MDA-kb2 – luciferase reporter gene assay	0.1 pM to 10 μM	Antagonist	Ma M et al. (2022)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 896 ± 68.3	Liu X et al. (2019)	
		HELN-PR – luciferase reporter gene assay	1 nM to 10 μM	Antagonist; IC <sub>50</sub> (μM) 7.18 ± 3.69	Grimaldi et al. (2019)	
	GR	PR-CALUX	10 nM to 10 μM	No effect	Šauer et al. (2021)	
		MCF7 – gene expression	1 μM	Induction	Pelch KE et al. (2019)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 213 ± 8.98	Liu X et al. (2019)	
	GR	HMLN-GR – luciferase reporter gene assay	1 nM to 10 μM	Antagonist; IC <sub>50</sub> (μM) 16.1 ± 5.7	Grimaldi et al. (2019)	
		CHO-K1 – luciferase reporter gene assay	100 nM to 10 μM	Antagonist; RIC <sub>50</sub> 5.6 μM	Kojima et al. (2019)	

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Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
BPS-MAE	PPAR $\gamma$	MDA-kb2 – luciferase reporter gene assay	0.1 pM to 10 $\mu$ M	Agonist; logLOEC –9.49 (M)	Chen Q et al. (2020)
		CHO-K1 – luciferase reporter gene assay		Antagonist; logRIC <sub>20</sub> –7.75 (M)	Chen Q et al. (2020)
		MDA-kb2 – luciferase reporter gene assay		Antagonist	Ma M et al. (2022)
	PXR	Competitive receptor binding affinity assay		Inert	Liu X et al. (2019)
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 2450 $\pm$ 194	Liu X et al. (2019)
	ER $\alpha$	HG5LN-PXR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 19 $\pm$ 2.3; max efficacy 44%	Grimaldi et al. (2019)
		COS-7 – luciferase reporter gene assay	100 nM to 10 $\mu$ M	Agonist; REC <sub>20</sub> 6.3 $\mu$ M	Kojima et al. (2019)
	ER $\beta$	Competitive fluorescence polarization binding assay		No effect	Keminer et al. (2020)
		HepG2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)
		Competitive fluorescence polarization binding assay		Inactive	Keminer et al. (2020)
HepG2 – luciferase reporter gene assay		3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)	
AR	Competitive fluorescence polarization binding assay		No effect	Keminer et al. (2020)	
	MDA-kb2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)	
BPZ	ER $\alpha$	Competitive binding affinity assay		IC <sub>50</sub> (nM) 57.5 $\pm$ 5.48	Liu X et al. (2019)
		Binding (cell-free system)	1 nM to 100 $\mu$ M	Kd (nM) 939 $\pm$ 198	Böckers et al. (2020)
		Competitive fluorescence polarization binding assay		IC <sub>50</sub> 0.08 $\mu$ M	Keminer et al. (2020)
	ER $\beta$	Competitive binding affinity assay		IC <sub>50</sub> (nM) 61.8 $\pm$ 6.66	Liu X et al. (2019)
		MVLN cells, ER $\alpha$ binding affinity	1 nM–100 $\mu$ M	Agonist; EC <sub>50</sub> 11 nM; max induction 58.6% (vs. E2)	Lin et al. (2021)
	ER $\alpha$	MCF7 – luciferase reporter gene assay	10 pM to 100 $\mu$ M	Agonist; AC <sub>50</sub> 0.4 $\mu$ M	Mesnage et al. (2017)
		Yeast two-hybrid assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> 8.63 $\mu$ M	Conroy-Ben et al. (2018)
	ER $\alpha$	HELN-ER $\alpha$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.124 $\pm$ 0.30; max efficacy 45%	Grimaldi et al. (2019)
		CHO-K1 – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 97 nM	Kojima et al. (2019)
	ER $\beta$	HepG2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 0.4 $\mu$ M; max efficacy 101.7%	Pelch KE et al. (2019)
		Yeast two-hybrid assay	10 pM to 10 $\mu$ M	Agonist; EC <sub>50</sub> 6 $\mu$ M	van Leeuwen et al. (2019)
	ER $\beta$	HEK-ESR1 over-expressing cells	25 $\mu$ M (4 and 8 h)	Inactivation: –0.0510 (4 h); –0.13 (8 h)	Böckers et al. (2020)
		GeneBLAzer transactivation assay	10 fM to 10 $\mu$ M	Agonist; EC <sub>50</sub> 3.7 pM	Keminer et al. (2020)
	ER $\alpha$	MVLN – luciferase reporter gene assay		Agonist; logREC <sub>20</sub> –6.86 (M)	Chen Q et al. (2020)
		HeLa – luciferase reporter gene assay	10 pM to 10 $\mu$ M	Agonist; EC <sub>50</sub> (nM) 102 $\pm$ 7.99	Liu X et al. (2021)
	ER $\beta$	MCF7 – proliferative potential (E-screen assay)	10 pM to 100 $\mu$ M	Induction; AC <sub>50</sub> 0.11 $\mu$ M	Mesnage et al. (2017)
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 128 $\pm$ 14.1	Liu X et al. (2019)
		Competitive fluorescence polarization binding assay		IC <sub>50</sub> 1.1 $\mu$ M	Keminer et al. (2020)
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 132 $\pm$ 10.6	Liu X et al. (2021)
HELN-ER $\beta$ – luciferase reporter gene assay		1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.088 $\pm$ 0.03; max efficacy 53%	Grimaldi et al. (2019)	
CHO-K1 – luciferase reporter gene assay		1 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 18 nM	Kojima et al. (2019)	
HepG2 – luciferase reporter gene assay		3 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 0.5 $\mu$ M; max efficacy 61.3%	Pelch KE et al. (2019)	
ER $\beta$	GeneBLAzer transactivation assay	10 fM to 10 $\mu$ M	Inactive	Keminer et al. (2020)	

(continued)



Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference	
ERR $\gamma$		HeLa – luciferase reporter gene assay	10 pM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> (nM) 1160 $\pm$ 61.6	Liu X et al. (2021)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 13.4 $\pm$ 1.06	Liu X et al. (2019)	
AR		HG5LN-ERR $\gamma$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 5.24 $\pm$ 0.84; max efficacy 115%	Grimaldi et al. (2019)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 6680 $\pm$ 139	Liu X et al. (2019)	
		Competitive fluorescence polarization binding assay		IC <sub>50</sub> 1.6 $\mu$ M	Keminer et al. (2020)	
		MDA-kb2 – luciferase reporter gene assay	50 $\mu$ M	Antagonist	Kolšek et al. (2015)	
		Yeast two-hybrid assay	1 nM to 10 $\mu$ M	No effect	Conroy-Ben et al. (2018)	
		HELN-AR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> (nM) 3.58 $\pm$ 1.78	Grimaldi et al. (2019)	
		CHO-K1 – luciferase reporter gene assay	100 nM to 10 $\mu$ M	Antagonist; RIC <sub>50</sub> 7.3 $\mu$ M	Kojima et al. (2019)	
		MDA-kb2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	Antagonist; REC <sub>50</sub> 1.3 $\mu$ M; max efficacy 80.7%	Pelch KE et al. (2019)	
		Yeast two-hybrid assay	10 pM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> 60 $\mu$ M	van Leeuwen et al. (2019)	
		GeneBLAzer transactivation assay	10 fM to 10 $\mu$ M	inactive	Keminer et al. (2020)	
PR		MDA-kb2 – luciferase reporter gene assay	0.1 pM to 10 $\mu$ M	Antagonist	Ma M et al. (2022)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 1890 $\pm$ 158	Liu X et al. (2019)	
		HELN-PR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> ( $\mu$ M) 7.64 $\pm$ 3.22	Grimaldi et al. (2019)	
GR		MCF7 – gene expression	1 $\mu$ M	Induction	Pelch KE et al. (2019)	
		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 226 $\pm$ 9.89	Liu X et al. (2019)	
PPAR $\gamma$		MDA-kb2 – luciferase reporter gene assay	50 $\mu$ M	Antagonist; IC <sub>50</sub> 22 $\mu$ M	Kolšek et al. (2015)	
		HMLN-GR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)	
		CHO-K1 – luciferase reporter gene assay	100 nM to 10 $\mu$ M	Antagonist; RIC <sub>50</sub> 8.8 $\mu$ M	Kojima et al. (2019)	
		MDA-kb2 – luciferase reporter gene assay		Agonist; logLOEC –5.70 (M); antagonist; logRIC <sub>20</sub> –8.85 (M)	Chen Q et al. (2020)	
		CHO-K1 – luciferase reporter gene assay		No effect	Chen Q et al. (2020)	
		MDA-kb2 – luciferase reporter gene assay	0.1 pM to 10 $\mu$ M	Antagonist	Ma M et al. (2022)	
		Competitive receptor binding affinity assay		Inert	Liu X et al. (2019)	
	PXR		Competitive receptor binding affinity assay		IC <sub>50</sub> (nM) 2150 $\pm$ 53.3	Liu X et al. (2019)
			HG5LN-PXR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 10.2 $\pm$ 0.98; max efficacy 58%	Grimaldi et al. (2019)
	TBBPA		COS-7 – luciferase reporter gene assay	100 nM to 10 $\mu$ M	Agonist; REC <sub>50</sub> 8.7 $\mu$ M	Kojima et al. (2019)
ER $\alpha$		MCF7 – competitive binding with 17 $\beta$ -estradiol	10 nM to 100 $\mu$ M	Relative binding affinity (vs. E2) = 0.004%	Samuelson et al. (2001)	
		MCF7 – competitive binding with 17 $\beta$ -estradiol	100 nM to 1 mM	Relative binding affinity (vs. E2) = 0.013%; EC <sub>50</sub> (nM) 250 $\pm$ 129	Olsen et al. (2003)	
		ER $\alpha$ affinity column	1 nM	No activity	Riu, Le Maire, et al. (2011)	
		MCF7 – luciferase reporter gene assay		Agonist; EC <sub>50</sub> 19 $\mu$ M	Kitamura et al. (2005)	
		ER-CALUX assay		No effect	Hamers et al. (2006)	
		Yeast two-hybrid assay	1 nM to 100 $\mu$ M	No effect	Li J et al. (2010)	
		OECD TG 455 – STTA assay on hER $\alpha$ -HeLa-9903 cells	10 <sup>-5</sup> to 10 <sup>-10</sup> M	No effect	Lee HK et al. (2012)	
		MELN – luciferase reporter gene assay	0.01–10 $\mu$ M	No effect	Molina-Molina et al. (2013)	
		Bioluminescence yeast estrogen screen assay		No effect	Ruan et al. (2015)	
		HELN-ER $\alpha$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)	

(continued)

Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
		HepG2 – luciferase reporter gene assay	3 nM to 10 µM	No effect	Pelch KE et al. (2019)
		Yeast two-hybrid assay	10 pM to 10 µM	Antagonist; IC <sub>50</sub> 0.6 µM	van Leeuwen et al. (2019)
		OECD TG 455 – STTA assay on hERα-HeLa-9903 cells	10 nM to 10 µM	No effect	Durcik, Hiti, et al. (2022)
		MCF7 – proliferative potential (E-screen assay)	10 nM to 100 µM	Relative proliferative potency (vs. E2) > 0.000003	Samuelsen et al. (2001)
		MCF7 – proliferative potential (E-screen assay)	100 nM to 1 mM	No effect	Olsen et al. (2003)
		MCF7 – proliferative potential (E-screen assay)	10 nM to 10 µM	No effect	Molina-Molina et al. (2013)
		MCF7 – proliferation assay		Agonist; EC <sub>20</sub> 124 µM; antagonist; IC <sub>20</sub> 451.2 µM	Krivoshiev et al. (2016)
		MCF7 – protein expression	30 µM	No effect	Olsen et al. (2003)
	ERβ	3T3-L1 cells – gene expression	1 pM to 10 µM	Induction; 1 pM	Chappell et al. (2018)
		HELN-ERβ – luciferase reporter gene assay	1 nM to 10 µM	No effect	Grimaldi et al. (2019)
		HepG2 – luciferase reporter gene assay	3 nM to 10 µM	No effect	Pelch KE et al. (2019)
	ERRγ	3T3-L1 cells – gene expression	1 pM to 10 µM	Induction; 100 pM to 10 µM	Chappell et al. (2018)
		Two-hybrid yeast assay	1 nM to 1 µM	No effect	Li J et al. (2010)
		HG5LN-ERRγ – luciferase reporter gene assay	1 nM to 10 µM	No effect	Grimaldi et al. (2019)
	GPER	3T3-L1 cells – gene expression	1 pM to 10 µM	Induction; 100 pM	Chappell et al. (2018)
		OVCAR-3 proliferation assay	1, 10, and 50 nM (48 and 72 h)	Induction; 10 and 50 nM; max efficacy 131% (72 h)	Hoffmann et al. (2017)
		KGN proliferation assay	1, 10, and 50 nM (48 and 72 h)	Induction; 10 and 50 nM; max efficacy 127% (72 h)	Hoffmann et al. (2017)
		OVCAR-3/KGN gene expression	1, 10, and 50 nM (48 and 72 h)	No effect	Hoffmann et al. (2017)
		3T3-L1 cells – gene expression	1 pM to 10 µM	Induction; 1 pM to 10 µM	Chappell et al. (2018)
		MA-10 protein expression	10 nM	Induction	Gorowska-Wojtowicz et al. (2019)
	AR	Competitive binding assay	1–10 µM	Antagonist; max effect 62%	Beck et al. (2016)
		NIH3T3 – luciferase reporter gene assay	100 nM to 10 µM	No effect	Kitamura et al. (2005)
		AR-CALUX assay		No effect	Hamers et al. (2006)
		Two-hybrid yeast assay	1 nM to 100 µM	No effect	Li J et al. (2010)
		PALM – luciferase reporter gene assay	10 nM to 10 µM	No effect	Molina-Molina et al. (2013)
		Yeast receptor bioassay	10 pM to 100 µM	Antagonist; IC <sub>50</sub> 982 nM	Roelofs et al. (2015)
		HEK-293 – luciferase reporter gene assay	10 µM	Antagonist; 37% reduction of AR activation	Beck et al. (2016)
		MDA-kb2 – luciferase reporter gene assay	3 nM to 10 µM	No effect	Pelch KE et al. (2019)
		HELN-AR – luciferase reporter gene assay	1 nM to 10 µM	No effect	Grimaldi et al. (2019)
		Yeast two-hybrid assay	10 pM to 10 µM	Antagonist; IC <sub>50</sub> 0.4 µM	van Leeuwen et al. (2019)
		MCF7 AR1 – anti-proliferative potential	10 nM to 10 µM	No effect	Molina-Molina et al. (2013)
	PR	PR-CALUX		No effect	Hamers et al. (2006)
		Two-hybrid yeast assay	1 nM to 100 µM	Antagonist; RIC <sub>20</sub> 78 nM	Li J et al. (2010)
		HELN-PR – luciferase reporter gene assay	1 nM to 10 µM	No effect	Grimaldi et al. (2019)
		MCF7 – protein expression	30 µM	Induction	Samuelsen et al. (2001)
		MCF7 – protein expression	30 µM	Induction	Olsen et al. (2003)
	GR	Competitive binding assay		No effect	Beck et al. (2016)
		Competitive binding assay	1 nM to 100 µM	Agonist; EC20 (µM) 0.5 ± 1.1	Liu QS et al. (2020)
		Yeast receptor bioassay	10 pM to 100 µM	Antagonist; IC <sub>50</sub> 22 nM	Roelofs et al. (2015)
		HEK-293 – luciferase reporter gene assay		No effect	Beck et al. (2016)
		COS7 – luciferase reporter gene assay	10 nM to 10 µM	No effect	Chappell et al. (2018)
		HMLN-GR – luciferase reporter gene assay	1 nM to 10 µM	No effect	Grimaldi et al. (2019)
	PPARγ	3T3-L1 cells – gene expression	1 pM to 10 µM	Induction; 1–100 pM	Chappell et al. (2018)
		HGELN-PPARγ – competitive binding	10 nM to 10 µM	IC <sub>50</sub> 0.7 µM	Riu, Le Maire, et al. (2011)

(continued)

Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
TCBPA	PPAR $\alpha$ / $\beta$ / $\delta$	PPAR $\gamma$ affinity column	1 nM	Eluted fraction 97.5 $\pm$ 0.6%	Riu, Grimaldi, et al. (2011)
		Competitive binding assay	1 nM to 50 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 5.7 $\pm$ 0.9	Liu QS et al. (2020)
		HepG2 – luciferase reporter gene assay	10 nM to 10 $\mu$ M	Agonist	Akiyama et al. (2015)
		COS-7 cells-mPPRE-luciferase reporter gene assay	0.1 nM to 400 $\mu$ M	Agonist; EC <sub>50</sub> 3.9 $\mu$ M	Watt and Schlezinger (2015)
		COS7 – luciferase reporter gene assay (mPPAR $\gamma$ )	10 nM to 10 $\mu$ M	Agonist; AC <sub>50</sub> 397 $\mu$ M	Chappell et al. (2018)
		COS-7-hPPRE – luciferase reporter gene assay	10–20 $\mu$ M	Agonist	Andrews et al. (2020)
		HEK293 – luciferase reporter gene assay		Agonist; EC <sub>20</sub> ( $\mu$ M) 7.7 $\pm$ 1.1	Liu QS et al. (2020)
		HG5LN cell – luciferase reporter gene assay		EC <sub>50</sub> ( $\mu$ M) 0.2 $\pm$ 0.1	Garoché et al. (2021)
		Steatotic FaO rat hepatoma cells – gene expression	1 $\mu$ M	Induction	Grasselli et al. (2014)
		Mouse neocortical neurons – protein expression	10 $\mu$ M	Repression	Wojtowicz et al. (2014)
		3T3-L1 – gene expression	10 $\mu$ M	Induction	Akiyama et al. (2015)
		JEG-3 human placental cells – protein expression	10 nM and 10 $\mu$ M (24–72 h)	Induction; 10 nM, 10 $\mu$ M (24 h), 10 nM (48 h)	Honkisz and Wójtowicz (2015b)
		Bone marrow cells – gene expression	10 and 20 $\mu$ M (7 days)	Induction; 20 $\mu$ M	Watt and Schlezinger 2015
		3T3-L1 – gene expression	1 pM to 10 $\mu$ M (8 days)	Induction, 1 pM to 10 $\mu$ M	Chappell et al. (2018)
		hMSCs – gene expression	10 $\mu$ M	Induction	Kakutani et al. (2018)
		MA-10 protein expression	10 nM	Induction	Gorowska-Wojtowicz et al. (2019)
		Bone marrow cells – gene expression	20 $\mu$ M	No effect	Andrews et al. (2020)
		3T3-L1 cells – protein expression	10 and 20 $\mu$ M (10 days)	Induction	Liu QS et al. (2020)
		3T3-L1 – gene expression	20 $\mu$ M	Induction	Kim S et al. (2021)
		HepG2 transfected with PPAR $\gamma$ – protein expression	30 $\mu$ M	Induction	Cheng and Volz (2022)
		Fluorescence polarization competitive binding assay		Agonist; IC <sub>50</sub> ( $\mu$ M) 68 $\pm$ 11	Li CH et al. (2021)
		HGELN-PPAR $\alpha$ / $\beta$ – luciferase reporter gene assay		No effect	Riu, Grimaldi, et al. (2011) and Riu, Le Maire, et al. (2011)
		HEK293-PPAR $\beta$ / $\delta$ – luciferase reporter gene assay	0.1–50 $\mu$ M	Induction; 25 and 50 $\mu$ M	Li CH et al. (2021)
Steatotic FaO rat hepatoma cells – gene expression ( $\alpha$ )	1 $\mu$ M	Repression	Grasselli et al. (2014)		
Steatotic FaO rat hepatoma cells – gene expression ( $\beta$ / $\delta$ )	1 $\mu$ M	Induction	Grasselli et al. (2014)		
3T3-L1 – gene expression	20 $\mu$ M	Induction	Kim S et al. (2021)		
HG5LN-hPXR reporter cell line	10 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 11.97 $\pm$ 5.38	Molina-Molina et al. (2013)		
HG5LN-PXR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 123 $\pm$ 49.4; max efficacy 27%	Grimaldi et al. (2019)		
TCBPA	ER $\alpha$	MCF7 – competitive binding with 17 $\beta$ -estradiol	100 nM to 1 mM	Relative binding affinity (vs. E2) = 0.075%; EC <sub>50</sub> (2.8 $\pm$ 0.82) $\times$ 10 <sup>-6</sup> M	Olsen et al. (2003)
		ER $\alpha$ affinity column	1 nM	Active; eluted fraction 63.1 $\pm$ 4.7%	Riu, Le Maire, et al. (2011)
		MCF7 – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> 0.02 $\mu$ M	Kitamura et al. (2005)
		Two-hybrid yeast assay	1 nM to 100 $\mu$ M	No effect	Li J et al. (2010)
		HGELN-ER $\alpha$ stably transfected	10 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 0.53 $\pm$ 0.25	Riu, Grimaldi, et al. (2011)
		MELN – luciferase reporter gene assay	10 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 47.60 $\pm$ 5.26	Molina-Molina et al. (2013)
		Bioluminescence yeast estrogen screen assay		Agonist; EC <sub>50</sub> 2.53 $\mu$ M	Ruan et al. (2015)
		HELN-ER $\alpha$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 11.7 $\pm$ 1.7; max efficacy 70%	Grimaldi et al. (2019)
		HepG2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No significant effect	Pelch KE et al. (2019)

(continued)

Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
		OECD TG 455 – STTA assay on hER $\alpha$ -HeLa-9903 cells	10 nM to 50 $\mu$ M	Agonist; max effect 52.97% $\pm$ 2.79	Durcik, Hiti, et al. (2022)
		MCF7 – proliferative potential (E-screen assay)	100 nM to 1 mM	Relative proliferative potential (vs. E2) = 0.00003%	Olsen et al. (2003)
		MCF7 – proliferative potential (E-screen assay)	10 nM to 10 $\mu$ M	EC <sub>50</sub> 45.8 $\mu$ M	Molina-Molina et al. (2013)
		MCF7 – protein expression	10 $\mu$ M	No effect	Olsen et al. (2003)
		MCF7 – protein expression	10 nM, 100 nM, and 1 $\mu$ M (24 h)	Induction	Lei, Tang, et al. (2021)
		MCF7 – gene expression	1, 10, and 25 $\mu$ M (12 h)	Induction at 1 and 10 $\mu$ M; repression at 25 $\mu$ M	Lei, Tang, et al. (2021)
	ER $\beta$	HGELN-ER $\beta$ stably transfected	10 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 1.15 $\pm$ 0.51	Riu, Grimaldi, et al. (2011)
		HELN-ER $\beta$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 68 $\pm$ 17; max efficacy 21%	Grimaldi et al. (2019)
		HepG2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)
	ERR $\gamma$	Two-hybrid yeast assay	1 nM to 1 $\mu$ M	No effect	Li J et al. (2010)
		HG5LN-ERR $\gamma$ – luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)
	GPER	OVCAR-3 proliferation assay	1, 10, and 50 nM (48 and 72 h)	No effect	Hoffmann et al. (2017)
		KGN proliferation assay	1, 10, and 50 nM (48 and 72 h)	No effect	Hoffmann et al. (2017)
		OVCAR-3/KGN gene expression	1, 10, and 50 nM (48 and 72 h)	No effect	Hoffmann et al. (2017)
		MA-10 protein expression	1 nM	Repression	Gorowska-Wojtowicz et al. (2019)
		MCF7/SKBR3/MDA-MB-231 – protein expression	10 nM, 100 nM, and 1 $\mu$ M (24 h)	Induction	Lei, Tang, et al. (2021)
		MCF7 – gene expression	1, 10, and 25 $\mu$ M (12 h)	Induction at 1 and 10 $\mu$ M	Lei, Tang, et al. (2021)
		SKBR3 gene expression	10 nM to 10 $\mu$ M	Induction	Yu M et al. (2023)
	AR	NIH3T3 – luciferase reporter gene assay	100 nM to 10 $\mu$ M	Not significant; IC <sub>50</sub> 870 $\mu$ M	Kitamura et al. (2005)
		CV-1 – reporter gene assay	100 nM to 1010 $\mu$ M (+1 nM DHT)	Antagonist; IC <sub>50</sub> 10.45 $\mu$ M	Sun et al. (2006)
		CV-1 – reporter gene assay	100 nM to 1010 $\mu$ M (+100 nM DHT)	No effect	Sun et al. (2006)
		Two-hybrid yeast assay	1 nM to 100 $\mu$ M	Antagonist; RIC <sub>20</sub> 100 nM	Li J et al. (2010)
		PALM – luciferase reporter gene assay	10 nM to 10 $\mu$ M	No effect	Molina-Molina et al. (2013)
		MDA-kb2 – luciferase reporter gene assay	3 nM to 10 $\mu$ M	No effect	Pelch KE et al. (2019)
		HELN-AR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Antagonist; IC <sub>50</sub> ( $\mu$ M) 13.4 $\pm$ 3.7	Grimaldi et al. (2019)
		MCF7 AR1 – anti-proliferative potential	10 nM to 10 $\mu$ M	No effect	Molina-Molina et al. (2013)
	PR	Two-hybrid yeast assay	1 nM to 100 $\mu$ M	Antagonist; RIC <sub>20</sub> 27 nM	Li J et al. (2010)
		HELN-PR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)
		MCF7 – protein expression	10 $\mu$ M	Induction	Olsen et al. (2003)
		MCF7 – gene expression	1 $\mu$ M	No effect	Pelch KE et al. (2019)
		MCF7 – gene expression	1, 10, and 25 $\mu$ M (12 h)	Induction at 1, 10, and 25 $\mu$ M	Lei, Tang, et al. (2021)
		SKBR3 gene expression	10 nM to 10 $\mu$ M	Repression	Yu M et al. (2023)
	GR	HMLN-GR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	No effect	Grimaldi et al. (2019)
	PPAR $\gamma$	HGELN-PPAR $\gamma$ – competitive binding	10 nM to 10 $\mu$ M	IC <sub>50</sub> 6 $\mu$ M	Riu, Le Maire, et al. (2011)
		PPAR $\gamma$ affinity column	1 nM	Active; eluted fraction 84.7 $\pm$ 0.5%	Riu, Grimaldi, et al. (2011)
		HGELN-PPAR $\gamma$ – luciferase reporter gene assay	10 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 1.28 $\pm$ 0.16 $\mu$ M	Riu, Grimaldi, et al. (2011)
		HG5LN – luciferase reporter gene assay	10 pM to 10 $\mu$ M	EC <sub>50</sub> ( $\mu$ M) 0.3 $\pm$ 0.1	Garoche et al. (2021)
		MA-10 protein expression	1 nM	Repression	Gorowska-Wojtowicz et al. (2019)
	PPAR $\alpha$ / $\beta$ / $\delta$	Fluorescence polarization competitive binding assay (PPAR $\beta$ / $\delta$ )		Agonist; IC <sub>50</sub> ( $\mu$ M) 102 $\pm$ 15	Li CH et al. (2021)

(continued)



Table A1. Continued.

Compound	Receptor	Cell/model assay	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
PXR		HGELN-PPAR $\alpha$ / $\beta$ – luciferase reporter assay		No effect	Riu, Grimaldi, et al. (2011) and Riu, Le Maire, et al. (2011)
		HEK293-PPAR $\beta$ / $\delta$ – luciferase reporter gene assay	0.1–50 $\mu$ M	Induction; 50 $\mu$ M	Li CH et al. (2021)
		HG5LN-hPXR reporter cell line	10 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 8.49 $\pm$ 0.63 $\mu$ M	Molina-Molina et al. (2013)
		HG5LN-PXR – luciferase reporter gene assay	1 nM to 10 $\mu$ M	Agonist; EC <sub>50</sub> ( $\mu$ M) 24.1 $\pm$ 1.5; max efficacy 35%	Grimaldi et al. (2019)

AR: androgen receptor; BPE: bisphenol E; BPAP: bisphenol AP; BPP: bisphenol P; BPS-MAE: bisphenol S 4-allyl ether; BPZ: bisphenol Z; E2: 17 $\beta$ -estradiol; EC: effective concentration; ER $\alpha$ ; estrogen receptor  $\alpha$ ; ER $\beta$ ; estrogen receptor  $\beta$ ; ERR  $\gamma$ : estrogen-related receptor  $\gamma$ ; GPER: G protein-coupled estrogen receptor; GR: glucocorticoid receptor; IC: inhibitory concentration; OECD: Organisation for Economic Cooperation and Development; PPAR: peroxisome proliferator-activated receptor; PR: progesterone receptor; PXR: pregnane X receptor; REC: relative effective concentration; RIC: relative inhibitory concentration; TBBPA: tetra-bromo bisphenol A; TCBPA: tetra-chloro bisphenol A; TG: test guideline.

Row colors indicate the different assay types: yellow for competitive binding assays, green for reported gene and yeast assays, blue for proliferation assays, and orange for gene/protein expression assessment.

Table A2. Summary of the *in vivo*, *in vitro*, and *in chemico* assays reporting effects on thyroid hormone metabolism exerted by BPA analogues.

Evaluated markers	Experimental model	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
<b>BPA</b>				
T3	Rat – adult	200 mg/kg/d (35 d)	Inhibition	Mohammed et al. (2020)
	Rat – adult	40 mg/kg/d (15 d)	Induction	da Silva et al. (2019)
	Rat – adult – female	40 mg/kg/d (15 d)	No effect	da Silva et al. (2018)
	Rat – dams from PND 3 to PND 15 (breastmilk expo). Pups at PND 7 and 21	0.05 and 5 mg/kg/d	Dams: no effect. Female pups: no effect. Male pups: no effect	Santos-Silva et al. (2018)
	Rat – female – during gestation	0.01 and 0.05 mg/kg/d for gestation and lactation	No effect	Silva BS et al. (2019)
	Rat – pregnant and fetus exposed from GD0 to GD20	20 and 40 mg/kg/d	Dams and fetus: inhibition	Ahmed (2016)
	Rat – adult – maternal exposure	0.01 and 0.05 mg/kg/d for gestation and lactation	No effect	Silva BS et al. (2019)
	Ewe adult gestational exposure	5 mg/kg/d sc	Inhibition in dams and newborns	Viguié et al. (2013)
	Mouse CD-1 – pubertally exposed	0.25 and 25 mg/kg/d (4 w)	Inhibition	Jiang et al. (2016)
	Ewe gestational exposure	0.5–50 and 5000 $\mu$ g/kg/d sc gestation	Inhibition in gestating ewes at 50 $\mu$ g/kg/d no effect on late fetuses	Guignard et al. (2017)
T4	C57Bl6 mouse adult	0.002–0.02–20 mg/kg/d for male 0.002–0.02 mg/kg/d for female	No effect	Hu C et al. (2023)
	Rat – adult	200 mg/kg/d (35 d)	Inhibition	Mohammed et al. (2020)
	Rat	40 mg/kg/d (15 d)	No effect	da Silva et al. (2019)
	Rat – adult – female	40 mg/kg/d (15 d)	Induction	da Silva et al. (2018)
	Rat – dams and PND 7 and 21 pups, exposed since GD11	$4.4 \times 10^{-7}$ and $2.2 \times 10^{-4}$ M	Dams: inhibition only at $2.2 \times 10^{-4}$ M Female pups: no effect Male pups: induction at PND7 and inhibition at PND21	Xu X et al. (2007)
	Rat – dams from PND 3 to PND 15 (breastmilk expo) pups at PND 7 and 21	0.05 and 5 mg/kg/d	Dams: no effect Male pups: inhibition at 0.050 mg/kg/d Female pups: no effect	Santos-Silva et al. (2018)
	Rat – PND 9 pups exposed during gestation	0, 0.004, 0.04, or 0.4 mg/kg/d	No effect	Sadowski et al. (2014)
	Rat – female – during gestation	0.01 and 0.05 mg/kg/d for gestation and lactation	No effect	Silva BS et al. (2019)
	Rat – PND 15 pups, exposed since GD6	2.5, 25, 250, 2500, or 25,000 mg/kg/d	No effect	Bansal and Zoeller (2019)
	Rat – pregnant and fetus exposed from GD0 to GD20	20 and 40 mg/kg/d	Dams and fetus: inhibition	Ahmed (2016)
Rat – PND 20, exposed since GD6	4 and 40 mg/kg/d	No effect	Kobayashi et al. (2005)	
Rat – pups/maternal exposure	1, 10, and 50 mg/kg/d	Induction at P15	Zoeller et al. (2005)	

(continued)

Table A2. Continued.

Evaluated markers	Experimental model	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
	Rat – adult – maternal exposure	0.01 and 0.05 mg/kg/d for gestation and lactation	Inhibition: only for 0.01 mg/kg/day	Silva BS et al. (2019)
	Ewe adult gestational exposure	5 mg/kg/d sc	Inhibition in dams and newborns	Viguié et al. (2013)
	Mouse CD-1 – pubertally exposed	0.25 and 25 mg/kg/d (4 w)	Inhibition	Jiang et al. (2016)
	Ewe gestational exposure	0.5–50 and 5000 µg/kg/d sc gestation	Inhibition in gestating ewes at 50 and 5000 µg/kg for free T4 no effect on late fetuses	Guignard et al. (2017)
	C57Bl6 mouse adult	0.002–0.02–20 mg/kg/d for male 0.002–0.02 mg/kg/d for female	No effect	Hu C et al. (2023)
Tsh	Rat – adult	200 mg/kg/d (35 d)	Induction	Mohammed et al. (2020)
	Rat – adult – female	40 mg/kg/d (15 d)	Inhibition	da Silva et al. (2018)
	Rat – pups exposed during gestation up to PND9	0, 0.004, 0.04, or 0.4 mg/kg/d	No effect	Sadowski et al. (2014)
	Rat – pregnant and fetus exposed from GD0 to GD20	20 and 40 mg/kg/d	Dams and fetus: induction	Ahmed (2016)
	Rat – pups/maternal exposure	1, 10, and 50 mg/kg/d	No effect	Zoeller et al. (2005)
Pax8	Rat – GH3 cells – adult – female – pituitary	0 and $4.4 \times 10^{-8}$ , $4.4 \times 10^{-7}$ , $4.4 \times 10^{-6}$ , and $4.4 \times 10^{-5}$ M (2 d)	<i>Tshβ</i> expression: inhibition at 44 µM	Lee S et al. (2017)
	Rat – FRTL-5 cells – thyroid	$10^{-5}$ , $3 \times 10^{-4}$ , and $10^{-4}$ M (1 d/2 d)	Induction at 100 µM	Wu Y et al. (2016)
	Rat – FRTL-5 cells – thyroid	$10^{-9}$ , $10^{-8}$ , $10^{-7}$ , $10^{-6}$ $10^{-5}$ , and $10^{-4}$ M (1 d/3 d)	Induction, biphasic	Gentilcore et al. (2013)
Foxe1	Human – SKOV-3 cells – ovary	$10^{-8}$ , $10^{-7}$ , and $10^{-6}$ M (1 d)	Induction, biphasic	Gentilcore et al. (2013)
	Rat – FRTL-5 cells – thyroid	$10^{-5}$ , $3 \times 10^{-4}$ , and $10^{-4}$ M (1 d/2 d)	Induction at 100 µM	Wu Y et al. (2016)
Nkx2-1	Rat – FRTL-5 cells – thyroid	$10^{-9}$ , $10^{-8}$ , $10^{-7}$ , $10^{-6}$ $10^{-5}$ , and $10^{-4}$ M (1 d/3 d)	Induction, biphasic	Gentilcore et al. (2013)
	Rat – FRTL-5 cells – thyroid	0, $4.4 \times 10^{-8}$ , $4.4 \times 10^{-7}$ , $4.4 \times 10^{-6}$ , and $4.4 \times 10^{-5}$ M (1 d)	Induction, non-significant	Lee S et al. (2017)
	Rat – FRTL-5 cells – thyroid	$10^{-5}$ , $3 \times 10^{-4}$ , and $10^{-4}$ M (1 d/2 d)	No effect	Wu Y et al. (2016)
Tshr	Rat – FRTL-5 cells – thyroid	$10^{-9}$ , $10^{-8}$ , $10^{-7}$ , $10^{-6}$ $10^{-5}$ , and $10^{-4}$ M (1 d/3 d)	Induction, biphasic	Gentilcore et al. (2013)
	Rat – adult	200 mg/kg/d (35 d)	Inhibition of <i>tshr</i> expression	Mohammed et al. (2020)
	C57Bl6 mouse adult males	0.02–20 mg/kg/d for male	Decreased expression at 20 mg/kg no effect at 0.02	Hu C et al. (2023)
Nis	Rat – FRTL-5 cells – thyroid	0, $4.4 \times 10^{-8}$ , $4.4 \times 10^{-7}$ , $4.4 \times 10^{-6}$ , and $4.4 \times 10^{-5}$ M (1 d)	No effect	Lee S et al. (2017)
	Rat – FRTL-5 cells – thyroid	$10^{-9}$ , $10^{-8}$ , $10^{-7}$ , $10^{-6}$ $10^{-5}$ , and $10^{-4}$ M (1 d/3 d)	Induction	Gentilcore et al. (2013)
	Rat – adult	200 mg/kg/d (35 d)	Inhibition of <i>Slc5a5</i> expression	Mohammed et al. (2020)
	Rat – adult – female	40 mg/kg/d (15 d)	Inhibition I <sup>-</sup> uptake	da Silva et al. (2018)
	C57Bl6 mouse adult male	0.02–20 mg/kg/d for male	Decreased expression at both doses	Hu C et al. (2023)
	Rat – FRTL-5 cells – thyroid	0, $4.4 \times 10^{-8}$ , $4.4 \times 10^{-7}$ , $4.4 \times 10^{-6}$ , and $4.4 \times 10^{-5}$ M (1 d)	Induction: non-significant (2.5×-fold at 100 mg/L)	Lee S et al. (2017)
	Rat – FRTL-5 cells – thyroid	$10^{-8}$ , $10^{-7}$ , $10^{-6}$ , $10^{-5}$ , and $10^{-4}$ M (1 h)	Inhibition I <sup>-</sup> uptake (Ki $1.12 \times 10^{-4}$ M)	Wu Y et al. (2016)
Duox1	Rat – FRTL-5 cells – thyroid	$10^{-5}$ , $3 \times 10^{-4}$ , and $10^{-4}$ M (1 d/2 d)	Inhibition I <sup>-</sup> uptake at 10 and 3 µM for 48 and 24 h exposures, respectively	Wu Y et al. (2016)
	Rat – FRTL-5 cells – thyroid	$10^{-5}$ , $3 \times 10^{-4}$ , and $10^{-4}$ M (6 h/1 d/2 d)	<i>Slc5a5</i> expression: 6 h: no effect 24 h and 48 h: inhibition (100 µM)	Wu Y et al. (2016)
	Rat – FRTL-5 cells – thyroid	$10^{-9}$ , $10^{-8}$ , $10^{-7}$ , $10^{-6}$ $10^{-5}$ , and $10^{-4}$ M (1 d/3 d)	Induction	Gentilcore et al. (2013)
	Rat – PCCI3 cells – thyroid	$10^{-9}$ M	Inhibition	da Silva et al. (2018)
	Rat – PCCI3 cells – thyroid	$10^{-9}$ M	No effect	da Silva et al. (2018)
Duox2	Rat – PCCI3 cells – thyroid	$10^{-9}$ M	Induction	da Silva et al. (2018)
Tpo	Rat thyroid microsomes		No effect	Wu Y et al. (2016)

(continued)

Table A2. Continued.

Evaluated markers	Experimental model	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
Tg	Rat – adult Rat – adult – female C57Bl6 mouse adult male	10 <sup>-8</sup> , 10 <sup>-7</sup> , 10 <sup>-6</sup> , 10 <sup>-5</sup> , 10 <sup>-4</sup> , and 10 <sup>-3</sup> M (1 h) 200 mg/kg/d (35 d) 40 mg/kg/d (15 d) 0.02–20 mg/kg/d for male	Inhibition of <i>tpo</i> expression Inhibition Decreased expression at both doses	Mohammed et al. (2020) da Silva et al. (2018) Hu C et al. (2023)
	Rat – FRTL-5 cells – thyroid	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	Induction, non-significant	Lee S et al. (2017)
	Rat – FRTL-5 cells – thyroid	10 <sup>-5</sup> , 3 × 10 <sup>-4</sup> , and 10 <sup>-4</sup> M (6 h/1 d/2 d)	Tpo expression: 6 h: no effect 24 h and 48 h: inhibition (100 μM)	Wu Y et al. (2016)
	Rat – FRTL-5 cells – thyroid	10 <sup>-9</sup> , 10 <sup>-8</sup> , 10 <sup>-7</sup> , 10 <sup>-6</sup> 10 <sup>-5</sup> , and 10 <sup>-4</sup> M (1 d/3 d)	Induction, biphasic	Gentilcore et al. (2013)
	Rat – PCCI3 cells – thyroid Rat – FRTL-5 cells – thyroid	10 <sup>-9</sup> M 0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	Inhibition Induction, non-significant	da Silva et al. (2018) Lee S et al. (2017)
	Rat – FRTL-5 cells – thyroid	10 <sup>-5</sup> , 3 × 10 <sup>-4</sup> , and 10 <sup>-4</sup> M (6 h/1 d/2 d)	6 h: no effect 24 h and 48 h: induction at 100 and 30 μM, respectively	Wu Y et al. (2016)
	Rat – FRTL-5 cells – thyroid	10 <sup>-9</sup> , 10 <sup>-8</sup> , 10 <sup>-7</sup> , 10 <sup>-6</sup> 10 <sup>-5</sup> , and 10 <sup>-4</sup> M (1 d/3 d)	Induction	Gentilcore et al. (2013)
	MDCK cells – overexpressing human MCT8 gene	0, 7.8 × 10 <sup>-5</sup> , 1.5 × 10 <sup>-4</sup> , 3.1 × 10 <sup>-4</sup> , 6.3 × 10 <sup>-4</sup> , 1.3 × 10 <sup>-3</sup> , 2.5 × 10 <sup>-3</sup> , and 5 × 10 <sup>-3</sup> M	Inhibition T3 uptake at 125 and 250 μM	Dong and Wade (2017)
	MDCK cells – overexpressing human MCT8 gene or primary murine astrocytes	10 <sup>-5</sup> M	No effect on T3 uptake in either cell type	Johannes et al. (2016)
	TTR	<i>In chemico</i>	0–1.5 × 10 <sup>-4</sup> M	Binding affinity constant, 3.10 × 10 <sup>5</sup> M
	<i>In chemico</i> <sup>125</sup> I-T3 competitive binding	10 concentrations between 10 <sup>-11</sup> and 10 <sup>-5</sup> M	IC <sub>50</sub> = 1.67 × 10 <sup>-6</sup> M	Kudo et al. (2006)
	<i>In chemico</i>	6 concentrations in nM range	No binding	Marchesini et al. (2008)
	<i>In chemico</i>	8 conc. 2 × 10 <sup>-9</sup> to 5 × 10 <sup>-7</sup> M	No binding	Meerts et al. (2000)
	<i>In chemico</i> T4 competitive binding	8 conc. 5 × 10 <sup>-6</sup> to 5 × 10 <sup>-3</sup> M	IC <sub>50</sub> = 1.42 × 10 <sup>-4</sup> M	Šauer et al. (2021)
TBG	<i>In chemico</i>	0–1.5 × 10 <sup>-4</sup> M	Affinity constant 5.9 × 10 <sup>5</sup> L/M	Cao et al. (2011)
	<i>In chemico</i>	6 concentrations in nM range	No binding	Marchesini et al. (2008)
HSA	<i>In chemico</i>	0–10 <sup>-3</sup> M	Affinity constants at drug site I and site II are 2.90 × 10 <sup>4</sup> and 3.14 × 10 <sup>4</sup> L/mol	Cao et al. (2011)
Dio1	Rat liver tissue – DIO1 enzyme	5 × 10 <sup>-6</sup> , 5 × 10 <sup>-5</sup> , 4 × 10 <sup>-4</sup> , and 5 × 10 <sup>-3</sup> M for 1 h	Inhibition: EC <sub>50</sub> 1.8 × 10 <sup>-4</sup> M	da Silva et al. (2019)
	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	No effect	Lee S et al. (2017)
Dio2	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	No effect	Lee S et al. (2017)
	Rat adipose tissue – DIO2 enzyme	5 × 10 <sup>-6</sup> , 5 × 10 <sup>-5</sup> , 4 × 10 <sup>-4</sup> , and 5 × 10 <sup>-3</sup> M (3 d)	Inhibition: EC <sub>50</sub> 1.1 × 10 <sup>-3</sup> M	da Silva et al. (2019)
THR <sub>s</sub>	Two-hybrid yeast transactivation assay		Modest stimulation, EC <sub>50</sub> = 30.1 μM vs. 0.085 μM for T3	Lei et al. (2018)
	Human ovarian granulosa cell line	10 <sup>-9</sup> to 10 <sup>-7</sup> to 10 <sup>-5</sup> M	No effect on <i>thrα</i> expression	Mlynarcikova and Scsukova (2020)
	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	Inhibition at 44 μM both <i>thrα</i> and <i>β</i> expression	Lee S et al. (2017)
	<i>In chemico</i>	8 conc. 5 × 10 <sup>-6</sup> to 5 × 10 <sup>-3</sup> M	No agonistic or antagonistic effects on THR <sub>β</sub>	Šauer et al. (2021)
	Rat – GH3 cells – adult – female – pituitary. Proliferation assay	From 10 <sup>-9</sup> to 10 <sup>-6</sup> M	Weak stimulation of basal and T3-induced GH3 cell proliferation	Lee J et al. (2018)
	TSA201 human cells derived from embryonic kidney		Weak ligand of THR <sub>α</sub> and <i>β</i> Antagonist of THR <sub>α</sub> and <i>β</i>	Moriyama et al. (2002)

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Table A2. Continued.

Evaluated markers	Experimental model	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
	cells transfected with <i>thrs</i> and HEPG2 (endogenous TRs)	1 nM to 100 μM for TtSA cells 1 nM to 10 μM for HepG2	From 1 μM in transfected TSA201 cells for THR $\alpha$ 1 and from 100 nM for THR $\beta$ 1 Antagonist at 10 μM for endogenous TRs in HEPG2	
<b>BPAP</b>				
<i>Tsh<math>\beta</math></i>	Rat – GH3 cells – adult – female – pituitary	0.034, 0.34, 3.4, and 34 μM (2 d)	No effect	Lee S et al. (2017)
<i>Pax8</i>	Rat – FRTL-5 cells – thyroid	0.34, 3.4, and 34 μM (1 d)	No effect	Lee S et al. (2017)
<i>Nkx2.1</i>	Rat – FRTL-5 cells – thyroid	0.34, 3.4, and 34 μM (1 d)	No effect	Lee S et al. (2017)
<i>Tshr</i>	Rat – FRTL-5 cells – thyroid	0.34, 3.4, and 34 μM (1 d)	No effect	Lee S et al. (2017)
<i>Slc5a5</i>	Rat – FRTL-5 cells – thyroid	0.34, 3.4, and 34 μM (1 d)	No effect	Lee S et al. (2017)
<i>Tpo</i>	Rat – FRTL-5 cells – thyroid	0.34, 3.4, and 34 μM (1 d)	No effect	Lee S et al. (2017)
<i>Tg</i>	Rat – FRTL-5 cells – thyroid	0.34, 3.4, and 34 μM (1 d)	No effect	Lee S et al. (2017)
<i>Dio1</i>	Rat – GH3 cells – adult – female – pituitary	0.034, 0.34, 3.4, and 34 μM (2 d)	No effect	Lee S et al. (2017)
<i>Dio2</i>	Rat – GH3 cells – adult – female – pituitary	0.034, 0.34, 3.4, and 34 μM (2 d)	Inhibition at 34 μM	Lee S et al. (2017)
<i>Thrx</i>	Rat – GH3 cells – adult – female – pituitary	0.034, 0.34, 3.4, and 34 μM (2 d)	Inhibition at 34 μM	Lee S et al. (2017)
<i>Thrb</i>	Rat – GH3 cells – adult – female – pituitary	0.034, 0.34, 3.4, and 34 μM (2 d)	Inhibition at 34 μM	Lee S et al. (2017)
THRs	Rat – GH3 cells – adult – female – pituitary. proliferation assay	From 10 <sup>-9</sup> to 10 <sup>-6</sup> M	Weak stimulation of basal and T3-induced GH3 cell proliferation at 10 <sup>-6</sup> M after 96 h exposure	Lee S et al. (2017)
<b>BPE</b>				
TTR	<i>In chemico</i> TTR THR $\beta$ callux assay	8 conc. 5 × 10 <sup>-6</sup> to 5 × 10 <sup>-3</sup> M	Weak potency for T3 binding inhibition: IC <sub>50</sub> = 1.3 × 10 <sup>-4</sup> M	Šauer et al. (2021)
THRs	<i>In chemico</i> TTR THR $\beta$ callux assay <i>In chemico</i> hybrid yeast assay <i>THR<math>\alpha</math></i>	8 conc. 5 × 10 <sup>-6</sup> to 5 × 10 <sup>-3</sup> M	No agonistic or antagonistic activity on THR $\beta$ Stimulation with low potency = 69.9 μM (vs. 0.085 μM for T3)	Šauer et al. (2021) Lei et al. (2017)
<b>BPP</b>				
<i>Thrb</i>	Rat – GH3 cells – adult – female – pituitary	30–30 μM	Inhibition of <i>thrb</i> expression from 3 μM	Lee S et al. (2017)
<i>Tsh<math>\beta</math></i>	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	Inhibition, non-significant	Lee S et al. (2017)
<i>Pax8</i>	Rat – FRTL-5 cells – thyroid	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	No effect	Lee S et al. (2017)
<i>Nkx2.1</i>	Rat – FRTL-5 cells – thyroid	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	No effect	Lee S et al. (2017)
<i>Tshr</i>	Rat – FRTL-5 cells – thyroid	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	No effect	Lee S et al. (2017)
<i>Slc5a5</i>	Rat – FRTL-5 cells – thyroid	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	No effect	Lee S et al. (2017)
<i>Tpo</i>	Rat – FRTL-5 cells – thyroid	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	No effect	Lee S et al. (2017)
<i>Tg</i>	Rat – FRTL-5 cells – thyroid	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (1 d)	No effect	Lee S et al. (2017)
<i>Dio1</i>	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	Inhibition at 10 mg/L	Lee S et al. (2017)
<i>Dio2</i>	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	Inhibition at 10 mg/L	Lee S et al. (2017)
<i>Thrx</i>	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	No effect	Lee S et al. (2017)
<i>Thrb</i>	Rat – GH3 cells – adult – female – pituitary	0, 4.4 × 10 <sup>-8</sup> , 4.4 × 10 <sup>-7</sup> , 4.4 × 10 <sup>-6</sup> , and 4.4 × 10 <sup>-5</sup> M (2 d)	Inhibition	Lee S et al. (2017)

(continued)



Table A2. Continued.

Evaluated markers	Experimental model	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
THRs	Rat – GH3 cells – adult – female – pituitary. proliferation assay	From 10 <sup>-9</sup> to 10 <sup>-6</sup> M	Weak stimulation of basal and T3-induced GH3 cell proliferation at 10 <sup>-6</sup> M after 96 h exposure	Lee J et al. (2018)
<b>BPZ</b>				
<i>Tshβ</i>	Rat – GH3 cells – adult – female – pituitary. proliferation assay	From 10 <sup>-9</sup> to 10 <sup>-6</sup> M	Weak stimulation of basal and T3-induced GH3 cell proliferation at 10 <sup>-6</sup> M after 96 h exposure	Lee J et al. (2018)
<i>Pax8</i>	Rat – FRTL-5 cells – thyroid	0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<i>Nkx2.1</i>	Rat – FRTL-5 cells – thyroid	0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<i>Tshr</i>	Rat – FRTL-5 cells – thyroid	0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<i>Slc5a5</i>	Rat – FRTL-5 cells – thyroid	0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<i>Tpo</i>	Rat – FRTL-5 cells – thyroid	0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<i>Tg</i>	0.46, 4.6, and 46 μM	0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<i>Dio1</i>	Rat – GH3 cells – adult – female – pituitary	0.046, 0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<i>Dio2</i>	Rat – GH3 cells – adult – female – pituitary	0.046, 0.46, 4.6, and 46 μM	Inhibition at 46 μM	Lee S et al. (2017)
<i>Thrx</i>	Rat – GH3 cells – adult – female – pituitary	0.046, 0.46, 4.6, and 46 μM	Inhibition at 0.46 μM	Lee S et al. (2017)
<i>Thrβ</i>	Rat – GH3 cells – adult – female – pituitary	0.046, 0.46, 4.6, and 46 μM	Inhibition at 4.6 μM	Lee S et al. (2017)
<i>Tshβ</i>	Rat – GH3 cells – adult – female – pituitary	0.046, 0.46, 4.6, and 46 μM	No effect	Lee S et al. (2017)
<b>TBBPA</b>				
T3	Rat – adult	0, 30, 100, or 300 mg/kg/d (28d)	Males: induction BMDL <sub>10</sub> 124 mg/kg/d Females: no effect	EFSA (2011)
	Rat – adult	0, 30, 100, and 300 mg/kg/d (28d)	Males: induction Females: no effect BMDL of 123.8 mg/kg/d	Van der Ven et al. (2008)
	Rat – adult	0, 100, 300, and 1000 mg/kg/d (13 w)	No effect	Osimitz et al. (2016)
	Rat – adult	100, 300, and 1000 mg/kg/d (13 w)	No effect	EFSA 2011
	Rat – adult	250 mg/kg/d (5 d)	No effect	Sanders et al. (2016)
	Rat – adults – female and male – pups	10, 100, and 1000 mg/kg/d (12 w)	Inhibition only for adult males at highest conc.	EFSA (2011)
	Rat – pups from adults exposed 70 (m) and 14 (f) days prior to mating and through lactation	0, 3, 10, 30, 100, 300, 1000, or 3000 mg/kg/d	Female pups: Induction BMDL <sub>10</sub> 2.3 mg/kg/d	EFSA (2011)
	Rat – pups from GD10 to PND 20	0–100–1000–10,000 ppm in diet	Male pups: inhibition at PND for 100 and 1000 ppm No effect at PNW11	Saegusa et al. (2009)
	Rat – pups exposed in utero until PND21	3, 10, 30, 100, 300, 1000, and 3000 mg/kg/d	Female pups: induction, BMDL 2.3 mg/kg/d Male pups: no effect	Van der Ven et al. (2008)
	Rat adult and GSD21exposed during pregnancy and lactation	10–100–1000 mg/kg/d	Adult male: decreased at highest concentration Adult female: no effect Pups: no effect	Cope et al. (2015)
T4	Rat – adult	0, 30, 100, or 300 mg/kg/d (28 d)	Males: inhibition BMDL <sub>10</sub> 48 mg/kg/d Females: no effect	EFSA (2011)
	Rat – adult	0, 30, 100, and 300 mg/kg/d (28 d)	Males: inhibition Females: no effect BMDL of 48 mg/kg/d	Van der Ven et al. (2008)
	Rat – adult	0, 100, 300, and 1000 mg/kg/d (13 w)	Males: inhibition at 33 and 90 d. Not dose dep. Females: inhibition at 33 d. Not dose dep.	Osimitz et al. (2016)
	Rat – adult	100, 300, and 1000 mg/kg/d (13 w)	Inhibition at all conc	EFSA (2011)
	Rat – adult	250 mg/kg/d (5 d)	Inhibition	Sanders et al. (2016)
	Rat – adult – female and male Rat – pups	10, 100, and 1000 mg/kg/d (12 w)	Adult males: inhibition at 100 and 1000 mg/kg/day Pups both sexes: inhibition at 100 and 1000 mg/kg/day. Adult females: inhibition at 1000 mg/kg/day	EFSA (2011)

(continued)

Table A2. Continued.

Evaluated markers	Experimental model	Dose range (time)	Response; EC <sub>50</sub> /effect size	Reference
Tsh/TSH	Rat – adult and GSD21 pups exposed during pregnancy and lactation	10, 100, or 1000 mg/kg/d	Adult males and females: inhibition, dose dep. NOEL: 10 mg/kg/d Pups: inhibition	Cope et al. (2015)
	Rat – pups from adults exposed 70 (m) and 14 (f) days prior to mating and through lactation	0, 3, 10, 30, 100, 300, 1000, or 3000 mg/kg/d	Male pups: inhibition Female pups: inhibition BMDL10 16 mg/kg/d BMDL10 31 mg/kg/d	EFSA (2011)
	Rat – pups from GD10 to PND 20	0–100–1000–10,000 ppm in diet	No effect	Saegusa et al. (2009)
	Rat – pups exposed in utero until PND21	3, 10, 30, 100, 300, 1000, and 3000 mg/kg/d	Inhibition. BMDL 30.8 mg/kg/d (m) and 16.1 mg/kg/d (f)	Van der Ven et al. (2008)
	Rat – pregnant and fetuses	5 mg/kg/d from GD10 to 16 d (13 w)	No effect in dams or fetuses	Meerts et al. (1999)
	Rat – adult	0, 100, 300, and 1000 mg/kg/d (13 w)	No effect	Osimitz et al. (2016)
	Rat – adult	100, 300, and 1000 mg/kg/d (13 w)	No effect	EFSA (2011)
	Rat – adult	250 mg/kg/d (5 d)	Induction non-significant	Sanders et al. (2016)
	Rat – adults and pups	10, 100, and 1000 mg/kg/d (12 w)	No effect	EFSA (2011)
	Rat – adult and GSD21 pups exposed during pregnancy and lactation	10, 100, or 1000 mg/kg/d	No effects	Cope et al. (2015)
TTR	Rat – pups from GD10 to PND 20	0–100–1000–10,000 ppm in diet	No effect	Saegusa et al. (2009)
	Rat – pregnant and fetuses	5 mg/kg/d	Increased TSH concentration in fetuses	Meerts et al. (1999)
	<i>In chemico</i>	10 concentrations between 10 <sup>-11</sup> and 10 <sup>-5</sup> M	Binding affinity: IC <sub>50</sub> = 3.1 × 10 <sup>-9</sup> M	Kudo et al. (2006)
	<i>In chemico</i>	6 concentrations in nM range	Strong binding affinity: IC <sub>50</sub> = 1.9 × 10 <sup>-8</sup> M	Marchesini et al. (2008)
TBG	<i>In chemico</i>	8 conc. 2 × 10 <sup>-9</sup> to 5 × 10 <sup>-7</sup> nM	Strong binding affinity: IC <sub>50</sub> = 7.7 × 10 <sup>-9</sup> M	Meerts et al. (2000)
	<i>In chemico</i>	8 conc. 10 <sup>-10</sup> to 10 <sup>-5</sup> M	Binding affinity: IC <sub>50</sub> = 3 × 10 <sup>-8</sup> M	Šauer et al. (2021)
	<i>In chemico</i>	6 concentrations in nM range	No binding affinity	Marchesini et al. (2008)
THR <sub>α</sub>	<i>In chemico</i>	Seven different concentrations from 50 μM and twofold dilutions between two consecutive concentrations	Relative binding potency/T3 15 and 100-fold lower for THR <sub>α</sub> and β, respectively	Ren et al. (2020)
	GH3 cells, competitive inhibition of <sup>125</sup> I-T3 binding to nuclear fractions Basal and T3-induced cell growth and GH production	10 <sup>-7</sup> to 10 <sup>-4</sup> M	Weak binding to THR: concentration-dependent inhibition of <sup>125</sup> I-T3-binding from 10 <sup>-6</sup> to 10 <sup>-4</sup> M (10 <sup>-10</sup> for T3) Agonistic effect from 10 <sup>-6</sup> M Not antagonistic effect	Kitamura et al. (2002)
TCBPA				
THR <sub>α</sub>	<i>In chemico</i>	0–10 <sup>-4</sup> M	Induction	Jugan et al. (2007)
TTR	<i>In chemico</i>	10 concentrations between 10 <sup>-11</sup> and 10 <sup>-5</sup> M	IC <sub>50</sub> = 2.2 × 10 <sup>-9</sup> M	Kudo et al. (2006)
	<i>In chemico</i> Competitive binding assay	6 concentrations in nM range	IC <sub>50</sub> = 3.2 × 10 <sup>-8</sup> M	Marchesini et al. (2008)
	<i>In chemico</i> competitive binding assay	8 conc. 2 × 10 <sup>-9</sup> to 5 × 10 <sup>-7</sup> nM	Weak binding capacity: IC <sub>50</sub> = 1.1 × 10 <sup>-7</sup> M	Meerts et al. (2000)
TBG	<i>In chemico</i>	6 concentrations in nM range	No binding capacity	Marchesini et al. (2008)
THR <sub>α</sub>	GH3 cells, competitive inhibition of <sup>125</sup> I-T3 binding to nuclear fractions Basal and T3-induced cell growth and GH production	10 <sup>-7</sup> to 10 <sup>-4</sup> M	Weak binding to THR: concentration-dependent inhibition of <sup>125</sup> I-T3-binding from 10 <sup>-6</sup> to 10 <sup>-4</sup> M (10 <sup>-10</sup> for T3) Agonistic effect from 10 <sup>-5</sup> to 10 <sup>-4</sup> M No antagonistic effect	Kitamura et al. (2002)

BMDL: benchmark dose lower bound; BPA: bisphenol A; BPAP: bisphenol AP; BPE: bisphenol E; BPP: bisphenol P; BPZ: bisphenol Z; Dio: iodothyronine deiodinase; Duox: dual oxidase; EC: effective concentration; Foxe1: forkhead box protein E1; GD: gestational day; HSA: human serum albumin; IC: inhibitory concentration; MCT8: monocarboxylate transporter 8; Nkx2-1: NK homeobox protein 1; Nis: sodium/iodide symporter; Pax8: paired box 8; PND: post-natal day; Slc5a5: solute carrier family 5 member 5; T3: triiodothyronine; T4: thyroxine; TBBPA: tetra-bromo bisphenol A; TBG: thyroxine-binding globulin; TCBPA: tetra-chloro bisphenol A; Tg: thyroglobulin; THR: thyroid hormone receptor; Tpo: thyroperoxidase; Tsh: thyroid-stimulating hormone; Tshr: thyroid-stimulating hormone receptor; TTR: transthyretin.

Row colors indicate the different assay types: yellow for *in vivo* assays; blue for *in vitro* assays, and orange for enzyme/*in chemico* assays.

**Table A3.** Summary of the *in vitro* assays reporting effects on steroid hormone secretion and gene expression of steroidogenic enzymes.

Compound	Hormone/enzyme	Cell model/assay	Dose range/time	Response; EC <sub>50</sub> /effect size	Reference
<b>BPAP</b>	17β-estradiol	H295R adrenal cells	1.56, 6.25, and 25 nM (48 h)	No effect	Lin et al. (2021)
	Testosterone	H295R adrenal cells	1.56, 6.25, and 25 nM (48 h)	Decrease: 6.25 and 25 nM	Lin et al. (2021)
	CYP11A	H295R adrenal cells – gene expression	1.56, 6.25, and 25 nM (48 h)	No effect	Lin et al. (2021)
	StAR	H295R adrenal cells – gene expression	1.56, 6.25, and 25 nM (48 h)	No effect	Lin et al. (2021)
	CYP17	H295R adrenal cells – gene expression	1.56, 6.25, and 25 nM (48 h)	No effect	Lin et al. (2021)
	CYP19	H295R adrenal cells – gene expression	1.56, 6.25, and 25 nM (48 h)	No effect	Lin et al. (2021)
	3βHSD2	H295R adrenal cells – gene expression	1.56, 6.25, and 25 nM (48 h)	No effect	Lin et al. (2021)
<b>BPE</b>	Progesterone	H295R steroidogenesis assay	0.8–50 μM	Increase: EC <sub>50</sub> 18.2 μM (8.8–37.9, 95% CI); max efficacy 689%	Rosenmai et al. (2014)
	17α-OH progesterone	H295R steroidogenesis assay	0.8–50 μM	Increase: EC <sub>50</sub> 23.0 μM (14.9–35.7, 95% CI); max efficacy 198%	Rosenmai et al. (2014)
	DHEA	H295R steroidogenesis assay	0.8–50 μM	Decrease: EC <sub>50</sub> 0.5 μM (0.2–1.6, 95% CI); max efficacy 68%	Rosenmai et al. (2014)
	Androstenedione	H295R steroidogenesis assay	0.8–50 μM	Decrease: EC <sub>50</sub> 0.3 μM (0.1–0.9, 95% CI); max efficacy 73%	Rosenmai et al. (2014)
	Estrone	H295R steroidogenesis assay	0.8–50 μM	Increase: EC <sub>50</sub> 13.6 μM (6.2–29.7, 95% CI); max efficacy 226%	Rosenmai et al. (2014)
	17β-estradiol	H295R steroidogenesis assay	0.8–50 μM	Increase: EC <sub>50</sub> 22.2 μM ±0.7; max efficacy 212 ± 29%	Rosenmai et al. (2014)
	Testosterone	H295R steroidogenesis assay	0.8–50 μM	Decrease: EC <sub>50</sub> 5.0 μM (3.4–7.3, 95% CI); max efficacy 58%	Rosenmai et al. (2014)
	Cortisol	H295R steroidogenesis assay	0.8–50 μM	No effect	Rosenmai et al. (2014)
	Corticosterone	H295R steroidogenesis assay	0.8–50 μM	Increase: EC <sub>50</sub> 16.1 μM (10.6–24.6, 95% CI); max efficacy 292%	Rosenmai et al. (2014)
<b>BPP</b>	17β-estradiol	H295R adrenal cells	0.39, 1.56, and 6.25 nM (48 h)	No effect	Lin et al. (2021)
	Testosterone	H295R adrenal cells	0.39, 1.56, and 6.25 nM (48 h)	No effect	Lin et al. (2021)
<b>BPZ</b>	17β-estradiol	H295R adrenal cells	0.39, 1.56, and 6.25 nM (48 h)	No effect	Lin et al. (2021)
	Testosterone	H295R adrenal cells	0.39, 1.56, and 6.25 nM (48 h)	Decrease: 0.39 and 6.25 nM	Lin et al. (2021)
	CYP11A	H295R adrenal cells – gene expression	0.39, 1.56, and 6.25 nM (48 h)	Decrease: 6.25 nM	Lin et al. (2021)
	StAR	H295R adrenal cells – gene expression	0.39, 1.56, and 6.25 nM (48 h)	No effect	Lin et al. (2021)
	CYP17	H295R adrenal cells – gene expression	0.39, 1.56, and 6.25 nM (48 h)	No effect	Lin et al. (2021)
	CYP19	H295R adrenal cells – gene expression	0.39, 1.56, and 6.25 nM (48 h)	No effect	Lin et al. (2021)
	3βHSD2	H295R adrenal cells – gene expression	0.39, 1.56, and 6.25 nM (48 h)	No effect	Lin et al. (2021)
<b>TBBPA</b>	Progesterone	MA-10 mouse Leydig cells	10 μM (48 h)	Increase	Roelofs et al. (2015)
		JEG-3 human placental cells	1 nM to 50 μM (24–72 h)	Increase: 1–50 nM (48 h), 100 nM 1 μM (48–72 h), 10–50 μM (24–72 h); max efficacy threefold at 50 μM (72 h)	Honkisz and Wójtowicz (2015b)
	17α-OH progesterone	MA-10 mouse Leydig cells	10 μM (48 h)	Increase	Roelofs et al. (2015)

(continued)

Table A3. Continued.

Compound	Hormone/enzyme	Cell model/assay	Dose range/time	Response; EC <sub>50</sub> /effect size	Reference
	Androstenedione	MA-10 mouse Leydig cells	10 µM (48 h)	Increase	Roelofs et al. (2015)
	17β-estradiol	MA-10 mouse Leydig cells	10 nM	Decrease	Gorowska-Wojtowicz et al. (2019)
		JEG-3 human placental cells	1 nM to 50 µM (24–72 h)	Increase: 1 nM and 50 µM (72 h), 10 nM to 1 µM (24–72 h), 10 µM (48–72 h); max efficacy 26–41% at 72 h	Honkisz and Wójtowicz (2015a)
		Human placental explants	5 nM to 50 µM (16 h)	Decrease (0.7-fold at 5 µM)	Arita et al. (2018)
	Testosterone	MA-10 mouse Leydig cells	0–30 µM	Increase (46-fold at 30 µM)	Dankers et al. (2013)
		MA-10 mouse Leydig cells	10, 30, and 100 µM (48 h)	Increase (56-fold at 100 µM)	Roelofs et al. (2015)
		Human placental explants	5 nM to 50 µM (16 h)	Increase (6.09-fold at 500 nM)	Arita et al. (2018)
	11-Deoxycorticosterone	MA-10 mouse Leydig cells	10 µM (48 h)	Increase	Roelofs et al. (2015)
	β-hCG	JEG-3 human placental cells	1 nM to 50 µM (24–72 h)	Decrease: 1 nM (72 h), 10 nM, 100 nM to 10 µM (24–72 h), 50 nM, 50 µM (24 and 72 h); max efficacy 37% 50 µM (72 h)	Honkisz and Wójtowicz (2015b)
	CYP11A	MA-10 mouse Leydig cells	10 µM	Induction	Dankers et al. (2013)
		MA-10 mouse Leydig cells	10 µM	No effect	Roelofs et al. (2015)
	StAR	MA-10 mouse Leydig cells	10 µM	Induction	Dankers et al. (2013)
		MA-10 mouse Leydig cells	10 µM	No effect	Roelofs et al. (2015)
	CYP17	MA-10 mouse Leydig cells	10 µM	Induction	Dankers et al. (2013)
		MA-10 mouse Leydig cells	10 µM	No effect	Roelofs et al. (2015)
	3β-HSD	MA-10 mouse Leydig cells	10 µM	No effect	Dankers et al. (2013)
		MA-10 mouse Leydig cells	10 µM	No effect	Roelofs et al. (2015)
	17β-Hsd	MA-10 mouse Leydig cells	10 µM	No effect	Dankers et al. (2013)
		MA-10 mouse Leydig cells	10 µM	No effect	Roelofs et al. (2015)
	CYP19 (aromatase)	H295R adrenal cells	2.5 and 7.5 µM	No effect	Cantón et al. (2005)
		JEG-3 human placental cells – protein expression	10 nM, 100 nM, and 10 µM (24 h)	Increase: 100 nM and 10 µM	Honkisz and Wójtowicz (2015a)
		JEG-3 human placental cells – enzyme activity	10 nM, 100 nM, and 10 µM (24–72 h)	Increase: 10 nM (48–72 h), 100 nM, 10 µM (24–72 h); max efficacy 41% for 10 µM at 72 h	Honkisz and Wójtowicz (2015a)
	CYP21	H295R adrenal cells – gene expression		Induction	Song et al. (2008)
<b>TCBPA</b>					
	17β-estradiol	MA-10 mouse Leydig cells	1 nM	Decrease	Gorowska-Wojtowicz et al. (2019)

BPAP: bisphenol AP; BPE: bisphenol E; BPP: bisphenol P; BPZ: bisphenol Z; CI: confidence interval; CYP: cytochrome P450; DHEA: dehydroepiandrosterone; EC: effective concentration; HSD: hydroxysteroid dehydrogenase; StAR: steroidogenic acute regulatory protein; TBBPA: tetra-bromo bisphenol A; TCBPA: tetra-chloro bisphenol A.



**Table A4.** *In vivo* studies describing the neural effects of developmental exposure to BPA alternatives.

Exposure period	Species/sex	Exposure duration	Exposure route	Substances/ doses	Age at analyses	Behavioral analyses	Neuroanatomical, endocrine, and molecular analyses	Reference
	C57BL/6 mice Males and females	From 7 days prenatally to the end of lactation	Drinking water	BPAP: 0.4 mg/kg bw/d	Postnatal weeks 6 to 7	<p><i>Open-field test</i>: Reduced number of entries, speed, and distance traveled in the center in BPAP-exposed males and females.</p> <p><i>Elevated plus maze</i>: Reduced time spent in the open arms for BPAP-exposed females.</p> <p><i>Marble burying test</i>: Increased repetitive behaviors in BPAP-exposed males and females.</p> <p><i>Novel object recognition</i>: Reduced time spent in exploration of the novel object in BPAP-exposed males and females.</p> <p><i>Three chamber test</i>: Reduced sociability and social novelty for BPAP-exposed males and females.</p>	<p><i>Hematoxylin/eosin and Nissl staining</i> The number of surviving cells was reduced in the dentate gyrus (DG) and CA1 area of BPAP-exposed males and females.</p> <p><i>Omic analyses</i> RNAseq analysis in BPAP-exposed males: 501 differentially expressed genes (168 downregulated and 333 up-regulated), with an enrichment in pathways related to behavior, synaptic signaling, neuronal development, and migration. <i>Single nucleus RNA-seq of the hippocampus</i>: Among the cell types identified (neurons, astrocytes, microglia, oligodendrocytes, and oligodendrocyte progenitor cells), the proportion of differentially expressed genes was as following: astrocyte &gt; microglia &gt; neurons and OPC &gt; oligodendrocytes. In gene set enrichment analysis, the identified pathways were related to Parkinson and neurodegeneration pathways in all cell clusters. The expression of microglial (C1qc, Ctss, and Iba1) and inflammatory (Tnfa, Cxcl10, Cxcl11, Il10, etc.) markers was up-regulated, while gene expression of development associated genes (Il1rap1, Sgk3, Ncam2, Kirrel, and Fkbp5) was down-regulated in BPAP-exposed animals. <i>Immunohistochemistry</i> Increased Iba1-immunoreactivity in the hippocampus of BPAP-exposed animals. <i>General and reproductive parameters</i>: Unchanged maternal, pregnancy duration, litter size, and sex ratio.</p>	Wu X et al. (2023)
Prenatal/postnatal	Wistar rats Males and females	Prenatal exposure 10 wks for fathers, 2 wks for mothers. Then throughout gestation/lactation. Weaning at PND21	Oral	TBBPA: 37.5, 125, 375, 1250, 3750, 12,500, or 37,500 mg/kg diet equivalent to 3, 10, 30, 100, 300, 1000, and 3000 mg/kg bw	Since PND50	<p>Analyses at PND50–110: <i>Auditory response</i>: Evaluation of brainstem auditory evoked potentials (BAEPs). In TBBPA-exposed females, BAEP thresholds and wave IV latency were increased in the low frequency range. In male rats, thresholds were unaffected, but absolute latency of wave IV and interpeak latencies II–IV showed exposure-related increases at low frequencies. <i>Context and cue conditioned fear</i>: No statistically significant effects were found. Analyses at PND90–140: <i>Sweet preference</i>: No statistically significant effects were observed on saccharin intake.</p>	<p>Reduced body weight of dams and male and female offspring exposed to the highest TBBPA dose. Increased T4 levels in offspring of both sexes and reduced T3 levels in females.</p>	Lilienthal et al. (2008)

(continued)

Table A4. Continued.

Exposure period	Species/sex	Exposure duration	Exposure route	Substances/doses	Age at analyses	Behavioral analyses	Neuroanatomical, endocrine, and molecular analyses	Reference
	Sprague-Dawley rats	GD10 to PND20 (weaning at PND21)	Oral (diet)	TBBPA: 100, 1000, or 10,000 ppm	PND20 PND77		Increased cell count for Reelin-expressing interneurons in the hilus at the two highest doses at PND20. Increased cell count for NeuN-expressing interneurons in the hilus at the two highest doses at PND77. Unchanged GAD-67 immunoreactivity at PND20 or PND77, immunoreactivity of EphA5 (tyrosine kinase receptor involved in neurogenesis, neuronal migration, and plasticity) and Tacr3 (role in survival and function of dopaminergic neurons) at PND20. No effect on brain weight and thyroid hormone levels. No detectable effects were found for the following assessment. <i>Neurofunctional or neurobehavioral deficits. F1 generation:</i> Neurological evaluation (lacrimation, salivation, urination and defecation frequencies, piloerection, pupillary light and reflexes, presence or absence of convulsions, abnormal movement and stereotypes, abnormal secretions, posture, muscle tone, forelimb and hind limb grip strength, etc. <i>F2 generation:</i> Functional observation battery	Saegusa et al. (2012)
	Sprague-Dawley rats Males and females	GD0 to GD19	Oral gavage	TBBPA: 0, 10, 100, 300, or 1000 mg/kg bw/d	P and F1: TH levels F2:	neurobehavior and brain histology	Thinning of the brain parietal cortex in F2 animals at PND 11 at the maternal oral dose of 1000 mg/kg/d. Increased T3/T4 ratio in females of P generation at 1000 mg/kg, and males and females of F1 generation at 100 and 1000 mg/kg. No effects on plasmatic TSH. No effects on body weight or pubertal timing.	Rock et al. (2019)
Cope et al. (2015)	Wistar rat Males and females	Exp1: GD9 to PND21 Exp2: GD6 to PND21 then offspring dosed until PND90	Oral	TBBPA Exp1: 0.1 mg/kg bw/d Exp2: 0.1, 25, and 250 mg/kg bw/d	Adulthood	<i>Experiment 1:</i> No effect in the dark light box or elevated plus maze for both sexes. Increased wheel activity of females exposed to TBBPA at 0.1 mg/kg/d. <i>Experiment 2:</i> Reduced time spent and number of entries and increased latency to enter in the central zone of the OFT for PND90 males exposed to 25 mg/kg/d. Reduced number of entries in the open arms of the EPM for PND145 males exposed to 25 and 250 mg/kg/d. <i>Open field test (OFT) at PND21:</i> No effect on activity. <i>Sociability and social hierarchy at weeks 10–20:</i> The mean velocity was increased in the chamber of the stranger mouse. Less time with the familiar and novel mice. Reduced interest in social novelty. Effects on body weight by other endocrine disruptors (BDE-47 and BPS) affected social rank of exposed animals.	Kim B et al. (2015)	
	CD-1 male mice	GD8 to PND21	Oral	TBBPA: 0.2 mg/kg bw/d	PND21 to week 20		Body weight: unaffected by TBBPA exposure.	Kim B et al. (2015)
	Intranasal							Liu QS et al. (2018)

(continued)

Table A4. Continued.

Exposure period	Species/sex	Exposure duration	Exposure route	Substances/doses	Age at analyses	Behavioral analyses	Neuroanatomical, endocrine, and molecular analyses	Reference
	Sprague Dawley male rats	PND2 to PND23		TBBPA-bis(2-hydroxyethyl ether) 0.086 mg/kg or 0.86 mg/kg	Just after exposure	<i>Rotarod</i> : Reduced latency to fall for the two doses at constant and acceleration speed modes. <i>Open field test</i> : Lower total traveled distance, traveled distance in the center, moving velocity, number of entries in the central zone at both doses.	<i>Microarray analysis of the cerebellum</i> : Affected gene expression measured for the higher dose: 911 upregulated and 433 downregulated genes Hematoxylin/eosin staining: lighter staining of the area under the cortex, demyelinated white matter, reduced neural cell number, pyknotic nuclei in TBBPA-0.86.	
	NMRI male mice	A single oral dose at PND10	Oral (gavage)	TBBPA: 11.5 mg/kg bw	PND11 PND17		PND11: no effects of TBBPA on calcium/calmodulin-dependent protein kinase II (CamKII), synaptophysin and Tau protein levels in the hippocampus and cortex. PND17: decreased binding sites of the nicotinic ligand cytosine in the frontal cortex.	Viberg and Eriksson (2011)
	ddY male mice	PND21 Acute: 3 h before the behavioral tests	Oral	TBBPA: 0.1 mg/kg, 5 mg/kg, or 250 mg/kg bw	Pre-pubertal age	Open-field test: increased horizontal movement activities at 5 mg/kg. Contextual fear conditioning paradigm: more freezing behavior for mice treated with TBBPA (0.1 mg/kg or 5 mg/kg bw) than controls. Y-maze test: TBBPA (0.1 mg/kg bw) increased the spontaneous alternation behavior.	TBBPA levels determined using LC/ESI-MS/MS system: High amounts of TBBPA were detected in the striatum after treatment with 0.1 mg/kg or 5 mg/kg body weight TBBPA; nonspecific accumulation of TBBPA after treatment with 250 mg/kg bw.	Nakajima et al. (2009)
Prepubertal/pubertal	Kunming male mice	28 days: PND21 to PND49	Oral	TBBPA: 50 and 100 mg/kg bw/d	PND22 to PND28: MWM	<i>Morris water maze</i> : Trajectories shown but no quantification of the time spent in the target quadrant. <i>Novel object recognition</i> : No effect.	<i>Analyses in the hippocampus</i> Increased reactive oxygen species at the higher dose and reduced glutathione at both doses. Increased Bax and reduced Bcl2 at the higher dose. Reduced BDNF levels at both doses, reduced PSD-95 levels at the higher dose. Increased acetylcholine esterase levels at the higher dose, reduced acetylcholine transferase levels at both doses.	Wang J and Dai (2022)
	C57BL/6 male mice	2 weeks from PND42 to PND56	Oral	TBBPA: 20, 100, or 500 mg/kg	PND56: behavioral tests PND65: sacrifice	<i>Passive avoidance test</i> : TBBPA at 100 and 500 mg/kg reduced memory retention.	Neurogenesis in the DG: TBBPA at 100 and 500 mg/kg reduced hippocampal neurogenesis by decreasing the survival and neuronal differentiation of newly generated cells without affecting proliferation of NPCs. Increased GFAP and Iba-1 fluorescence at the two highest doses without neuronal death; reduced BDNF levels and p-CREB immune-density at 500 mg/kg.	Kim AH et al. (2017)

Bax: Bcl-2-associated X; Bcl2: B-cell lymphoma 2; BDNF: brain-derived neurotrophic factor; bw: body weight; C1q: complement C1q C chain; CREB: c-AMP response element-binding protein; Ctss: cathepsin S; Cxcl10: C-X-C motif chemokine ligand 10; Cxcl11: C-X-C motif chemokine ligand 11; Fkbp5: FK506 binding protein 5; GFAP: glial fibrillary acidic protein; Iba1: ionized calcium-binding adapter molecule 1; Il10: interleukin 10; Il1rap1: interleukin 1 receptor accessory protein like 1; Kirrel: Kirre like nephrin family adhesion molecule 1; LC/ESI-MS/MS: liquid chromatography electrospray ionization tandem mass spectrometry; Ncam2: neural cell adhesion molecule 2; NeuN: neuronal nuclear protein; p-CREB: phospho-CREB; PND: postnatal day; RNA-seq: RNA sequencing; Sgk3: serum/glucocorticoid regulated kinase; T3: triiodothyronine; T4: thyroxine; Tnfa: tumor necrosis factor  $\alpha$ ; wks: weeks.

Table A5. In vitro studies reporting the neural effects of BPA alternatives.

Substances and doses	Cell types	Analyses	Reference
BPA, BPS, BPF, BPE, BPB, BPZ, and BPAF All substances at 1, 10, and 100 nM in 0.01% DMSO	(i) Human embryonic stem cells (hESCs) (ii) hESC differentiated into neural epithelium (iii) neuron-like cells differentiated from adherent Neuronal Stem Cells	Cytotoxicity severity was assessed for all targeted compounds based on the IC <sub>50</sub> calculation or estimation; BPAF was defined as the most cytotoxic chemical tested whereas BPF and BPS the less cytotoxic. The results pointed out that BPA and its analogues did induce neurogenesis impairment (neural epithelium generation) at early stages of neurogenesis but did not exclude that the latter could interfere with a later stage of neurodevelopment. Gene expression related to axon guidance process was not modified by the exposure of BPA and its analogues; normalized values of the total and maximum- neurite length were down-regulated by BPA in a dose dependent manner. A significant reduction of total neurite length was noted for 4 out of the 6 BPA analogues tested (BPS, BPF, BPA, or BPAF) at 1 nM; while only 100 nM of BPB, BPZ, or BPE triggered a noticeable decrease in the total length of neurite.	Liang X et al. (2020)
BPA, BPE, BPAP, BPP, BPC, BPG, BPB, and BPPH All substances at 1 nM, 100 nM, and 10 µM for 3 h or 24 h.	IMR-32 neuroblastoma cell line	Treatment for 24 h: All bisphenols except BPPH increased ROS levels, since 1 nM (BPA, BPE) or 100 nM (BPC) or at 10 µM (BPG, BPAP, and BPP). The protein levels of Bax, Bak1, and caspase-3 were increased by 100 nM except for BPP and BPPH. The apoptosis rate was also increased since 100 nM (BPA and BPC) or at 10 µM (BPE, BPG, BPP, and BPPH). BPA, BPE, BPC, BPG, and BPAP at 10 µM and BPAP at 100 nM also significantly increased Ca <sup>2+</sup> levels in IMR-32 cells. Treatment for 3 h: BPA, BPE, BPC, BPAP, and BPP at 10 µM increased intracellular Ca <sup>2+</sup> levels in IMR-32 cells, suggesting an activation of GPER. This effect was attenuated by GPER antagonist (G15) treatment (except for BPP). The ROS levels induced by BPC and BPAP were also reduced by G15. Molecular dynamics simulation suggests that BPA, BPE, BPC, and BPAP may activate GPER.	Wang L et al. (2023)
TBBPA HEI-OC1 cells: 125 µg/mL of TBBPA for 6, 12, 18, and 24 h TBBPA Doses: 2, 5, and 10 µM BPA 10 and 15 µM 0.1% DMSO 24h incubation	-HEI-OC1 auditory cell line Corti organ explants from postnatal day 2 rats Primary cerebellar granule cells (CGC) – Wistar rats	Toxicity induced in HEI-OC1 auditory cell line. Increased percentage of apoptotic cells at 18 h and 24 h in Corto organ explants from postnatal day 2 rats. A significant increase of LDH was observed in TBBPA-treated cells (10 and 20 µM) compared to the control cells; LC50 values were estimated at 7 µM. The concentration levels of BPA of 5 and 15 µM did not induce CGC death. These results highlight that the cytotoxic effects observed <i>in vitro</i> seem to be related to the bromination of chemical tested. At the dose of 5 µM, TBBPA triggered nuclear morphology alteration in CGC, chromatin condensation yielding and 63% of apoptotic cells. TBBPA altered gene expression entailed in programmed neuronal death. The authors observed an absence of programmed necrosis induction, an early suppression of autophagy and anti-apoptotic genes, followed by a late activation of apoptosis-associated genes. TBBPA exposure induced, during the follow-up periods (3, 6, and 24 h), a gradual reduction in the % of living cells. The integrity of the plasma membrane was maintained, and a higher number of neurons with irregular shapes and shrinking nuclei was observed.	Park C et al. (2016) Reistad et al. (2007)
TBBPA Doses: 10 and 25 µM for 30 min Time points 3-, 6-, and 24-hour	Primary cerebellar granule cells (CGC) – Wistar rats	The authors observed an absence of programmed necrosis induction, an early suppression of autophagy and anti-apoptotic genes, followed by a late activation of apoptosis-associated genes. TBBPA exposure induced, during the follow-up periods (3, 6, and 24 h), a gradual reduction in the % of living cells. The integrity of the plasma membrane was maintained, and a higher number of neurons with irregular shapes and shrinking nuclei was observed.	Lenart et al. (2017)
TBBPA Thimerosal (TH), silver nanoparticles (INAg), valproic acid (VPA), and thalidomide (THAL)	Primary cerebellar granule cells (CGC) – Wistar rats	Levels of expression of SNAP-25 gene were reduced by 48 and 64% for VPA and Nag, respectively, and enhanced by 49% and 66% for TBBPA and THAL in comparison to controls. The SNAP-25 protein levels in CGCs were reduced by 73% for VPA and	Ziemińska et al. (2016)

(continued)



Table A5. Continued.

Substances and doses	Cell types	Analyses	Reference
Doses: TBBPA from 1 up to 100 $\mu\text{M}$ , THAL and VPA from 0.01 up to 5 mM 24 h exposure TBBPA Doses: 10 and 25 $\mu\text{M}$	Primary cerebellar granule cells (CGC) – Wistar rats	69% for TH, and enhanced by 21% for Nag, 25% for THAL and by 79% for TBBPA in comparison to controls.  Increase in intracellular concentration of zinc ions in primary CGC exposed to TBBPA. This increase was linked with an increase in its levels in the extracellular compartment and a decrease in the total cellular pool of zinc.  Alterations of in the mitochondrial membrane potential ( $\Delta\Psi\text{m}$ ) in CGC after TBBPA exposure. Decreased catalase activity after 30 min of incubation of CGC cultures with TBBPA.  CGC were depolarized by TBBPA exposure in primary culture and rat cerebellar slices.  TBBPA did not modify NMDA receptor radio-ligand binding to rat cortical membranes.  TBBPA induced glutamate release and depolarization mediated by AMPAR and NMDAR.  The tested chemicals were found to be cytotoxic at low concentrations (2.7–28 $\mu\text{M}$ depending on the compound). Intracellular levels of $[\text{Ca}^{2+}]$ and ROS were in these neuronal cells. The 3-targeted compounds induce cell death, at least in part by apoptosis via activation of caspases.  Rapid depolarization of mitochondria and cytochrome c release in these neuronal cells were induced by exposure to these BFRs. Cells exposed to HBCDD and TBBPA (1–5 $\mu\text{M}$ ), showed elevated levels of caspase 3/7 activity compared to control cells. Exposure to DBPE at 10 $\mu\text{M}$ also showed an increase in caspase 3/7 activity.  HBCDD induces mitochondrial depolarization of cells within a few seconds of exposure. This was observed by a fast decrease in rhodamine123 fluorescence in the cells. The study showed that HBCDD triggered an increase in A $\beta$ -42 levels in the extracellular compartment in a time- and dose-dependent manner. Significant levels of A $\beta$ -42 peptide were therefore measure in cells exposed to HBCDD for only 4 h in comparison to controls.  Exposure to TBBPA and DBPE at levels of 3–10 $\mu\text{M}$ for 12 h also showed a significant increase in A $\beta$ -42 concentrations in treated cells versus the control cells.  Deregulation of transcription factors determinant for neuronal development like ZIC1, ZIC3, HES3, IGFBR3 as well as DLX5 by the five targeted chemicals.  All the 5 chemicals targeted dysregulated genes (e.g. CNTN2, SLIT1, LRRC4C, RELN, CBLN1, CHRNB4, and GDF7) involved in axon growth/guidance and neuron transmission-related processes.  AhHR and WNT signaling pathways were impacted by all compounds. All five flame retardants significantly up-regulated CYP1A1 gene expression, suggesting that all of them could behave as agonists for the AhR signaling pathway.  Caspase-3 activation and apoptotic body formation were observed at concentration levels from 100 nM to 100 $\mu\text{M}$ of TBBPA.  TBBPA cytotoxic effects were highlighted at concentration levels between 50 nM and 100 $\mu\text{M}$ .  TBBPA did not trigger changes in cellular ROS levels. Nevertheless, an enhancement of fluorescence signal was noted in a cell-free model following TBBPA exposure (10–100 $\mu\text{M}$ ).	Ziemińska et al. (2017)
TBBPA Doses: from 7.5 to 25 $\mu\text{M}$	Primary cerebellar granule cells (CGC) – Wistar rats		Diamandakis et al. (2019)
TBBPA, DBPE, and HBCDD All substances: 0–30 $\mu\text{M}$ for up to 12 h	SH-SY5Y human neuroblastoma cells		Al-Mousa and Michelangeli (2012)
TCBPA, TBBPA, TBBPS, BDE-47, and BDE-209 BDE-209 doses: 10, 100, and 1000 nM TBBPA, TCBPA, TBBPS, BDE-47, as well as mix of 5 compounds: 0.01, 0.1, 1, and 5 $\mu\text{M}$	Human embryonic stem cell (HESC)		Liang S et al. (2019)
TBBPA Doses: 1 nM to 100 $\mu\text{M}$ at 30 min, 3, 6, and 24 h	Primary hippocampal neuron – Swiss mouse embryos (day 17–18 of gestation).		Szychowski and Wójtowicz (2016)

(continued)

Table A5. Continued.

Substances and doses	Cell types	Analyses	Reference
TBBPA, pentabromophenol (PBP), triphenyl phosphate (TPP) All substances at 10 and 40 µM in 0.0004% DMSO	BV-2 cells	Pro-inflammatory responses were impacted in a limited way. Mitochondrial respiration: (i) basal respiration was altered by PBP, (ii) ATP-related respiration was impacted by PBP and TBBPA, and (iii) maximal respiration by all 3 flame retardants. Basal glycolytic rate was impaired by PBP and TBBPA and compensatory glycolysis was altered by all three compounds. Phagocytosis was diminished by PBP and TBBPA. Direct and secondary activation of the NLRP3 inflammasome by PBP, aggregate formation.	Bowen et al. (2020)

Ach: acetylcholine; AMPAR:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; AhR: aryl hydrocarbon receptor; Bak1: BCL2 antagonist/killer 1; Bax: Bcl-2-associated X; BFR: brominated flame retardant; CGC: cerebellar granule cells; GPER: G protein-coupled estrogen receptor; HFR: halogenated flame retardant; hESCs: human embryonic stem cells; LOEC: the lowest observed effect concentration; NMDAR: N-methyl-D-aspartate receptor; ROS: reactive oxygen species.

Table A6. Summary of studies addressing the genotoxicity of the most commonly employed and data-rich BPA alternatives.

Substance	Cell model	Assay	Exposure conditions	Effect	Reference
<b>BPF</b>					
BPF	HepG2 cells	Comet assay	0.1, 1, and 10 $\mu\text{M}$ , for 4 and 24 h	Positive at $\geq 1 \mu\text{M}$ , for 4 h treatment	Fic et al. (2013)
	Hu-PBMC model	Micronucleus assay	0.625–10 $\mu\text{M}$ , for 48, 72, and 96 h	No cytotoxicity at 12.5, 25, 50, and 100 $\mu\text{M}$ , for 24 h exposure, for 72 and 96 h exposures	Yu M et al. (2023)
		$\gamma\text{-H2AX}$	0.625–10 $\mu\text{M}$ , for 48 h	Positive – mixture of BPA/BPF/BPS/BPAF, for 48 h exposure	Yu M et al. (2023)
	RWPE-1 cells	$\gamma\text{-H2AX}$	2.5, 5, 10, 15, and 20 $\mu\text{g}/\text{mL}$ for 24 and 72 h	Positive	Hercog et al. (2019)
		Comet assay	0.01–10 $\mu\text{g}/\text{mL}$	Negative	Mokra et al. (2017)
		Comet assay	0.0001–1 $\mu\text{g}/\text{mL}$	Cytotoxicity at 20 $\mu\text{g}/\text{mL}$ , for 72 h exposure, after 1 h and 4 h exposure	Mokra et al. (2017)
	RAD54 <sup>-/-</sup> mutant chicken DT40 cells	Fpg-modified comet assay		Positive	Kose et al. (2020)
		$\gamma\text{-H2AX}$	125 $\mu\text{M}$ , for 1 h	Positive (oxidative DNA damage) versus standard Comet assay	Lee S et al. (2013)
	S. Typhimurium strains TA98, TA100	Chromosomal aberration	31.3, 62.5, and 125 $\mu\text{M}$ , for 48 h	IC <sub>50</sub> = 65 $\mu\text{M}$	
		Ames test	4, 20, 100, and 500 $\mu\text{g}/\text{plate} \pm 59$	Negative	Fic et al. (2013)
<b>BPAF</b>					
BPAF	HepG2 cells	Comet assay	0.1, 1, and 10 $\mu\text{M}$ , for 4 and 24 h	Positive at $\geq 1 \mu\text{M}$ , for 4 h No cytotoxicity at 12.5, 25, 50, and 100 $\mu\text{M}$ , for 24 h	Fic et al. (2013)
	HepG2 3D spheroids	$\gamma\text{-H2AX}$	2.5, 5, 10, and 20 $\mu\text{g}/\text{mL}$ , for 24 and 72 h	Negative	Hercog et al. (2019)
		Comet assay	0.01, 0.1, 1, 10, and 40 $\mu\text{M}$ , for 24 h 0.01, 0.1, 1, and 10 $\mu\text{M}$ , for 96 h	No cytotoxicity at 2.5, 5, 10, and 20 $\mu\text{g}/\text{mL}$ , for 24 h Cytotoxicity at 20 $\mu\text{g}/\text{mL}$ , for 72 h	Sendra et al. (2023)
	Hu-PBMC model	$\gamma\text{-H2AX}$	0.01, 0.1, 1, 10, and 40 $\mu\text{M}$ , for 24 h 0.01, 0.1, 1, and 10 $\mu\text{M}$ , for 96 h	Positive	Mokra et al. (2017)
		Comet assay	0.01–10 $\mu\text{g}/\text{mL}$ , for 1 h and 4 h	Negative	Mokra et al. (2017)
	Hu-PBMC model	Comet assay	1 and 10 $\mu\text{g}/\text{mL}$ , for 1 h 0.1 and 1 $\mu\text{g}/\text{mL}$ , for 4 h	Positive	Mokra et al. (2018)
		$\gamma\text{-H2AX}$	0.001–1 $\mu\text{g}/\text{mL}$ , for 4 h and 48 h 50 $\mu\text{M}$ , for 1 h	Negative	Lee S et al. (2013)
	S. Typhimurium strains TA98, TA100	Chromosomal aberration	12.5, 25, and 50 $\mu\text{M}$ , for 48 h 4, 20, 100, and 500 $\mu\text{g}/\text{plate} \pm 59$	Negative	Fic et al. (2013)
	Germ cells of NMRI mice (wild-type) and OGG1-deficient C57/Bl6 mice	Chromosomal exchanges and aneuploidy. 8-Oxoguanine level (IF)	10 $\mu\text{M}$ in drinking water from 10.5 to 18.5 days postconception (dpc) (500 $\mu\text{g}/\text{kg}/\text{day}$ )	Increased reciprocal chromosomal exchanges, aneuploid oocytes and oxidative DNA damage in NMRI and OGG1KO mice (higher than WT).	Abdallah et al. (2023)
	<b>BPS</b>				
HepG2 cells	Comet assay	0.1, 1, and 10 $\mu\text{M}$ for 4 and 24 h	Positive at 1 $\mu\text{M}$ and above/4 h treatment	Fic et al. (2013)	
	$\gamma\text{-H2AX}$	2.5, 5, 10, and 20 $\mu\text{g}/\text{mL}$ , for 24 and 72 h	No cytotoxicity at 12.5, 25, 50, and 100 $\mu\text{M}$ for 24 h Negative	Hercog et al. (2019)	

(continued)

Table A6. Continued.

Substance	Cell model	Assay	Exposure conditions	Effect	Reference
HepG2 3D spheroids		$\gamma$ H2AX Micronucleus assay	0.625–10 $\mu$ M, for 48 h and 96 h 0.625–10 $\mu$ M, for 48, 72, and 96 h	Positive for 72 and 96 h exposure Positive – mixture of BPA/BPF/BPS/BPAF, for 48 h exposure	Yu M et al. (2023)
		Comet assay	0.01, 0.1, 1, 10, and 40 $\mu$ M for 24 h 0.01, 0.1, 1, and 10 $\mu$ M for 96 h	Positive Positive	Sendra et al. (2023)
		$\gamma$ H2AX	0.01, 0.1, 1, 10, and 40 $\mu$ M, for 24 h 0.01, 0.1, 1, and 10 $\mu$ M, for 96 h	Negative Positive	
BEAS-2B cells		Comet assay $\gamma$ -H2AX DNA damage response (DDR) activation (ATM, ATR, p53, Chk1, $\gamma$ -H2AX)	200 $\mu$ M, for 24 h 200 $\mu$ M for 24 h 200 $\mu$ M, for 24 h	Positive (but no quantitative data reported) Positive (less than 4 foci/nucleus, not biologically relevant) No DDR activation Cytotoxicity – positive; dose–response relationship at 12.5, 25, 50, 100, and 200 $\mu$ M for 24 h	George and Rupasinghe (2018)
Hu-PBMC model		Comet assay Comet assay – neutral version	0.01–10 $\mu$ g/mL 10 $\mu$ g/mL	Increased level of intracellular ROS (DCF assay), at 200 $\mu$ M for 24 h Positive after 1 h and 4 h exposure Positive, after 4 h exposure	Mokra et al. (2017)
Hu-PBMC model RWPE-1 cells		Comet assay Comet assay Fpg-modified comet assay	0.0001–1 $\mu$ g/mL 0, 50, 100, 200, 300, and 600 $\mu$ M, for 24 h	Positive after 4 h and 48 h exposure Positive Positive (oxidative DNA damage) IC <sub>50</sub> = 108 $\mu$ M Positive	Mokra et al. (2018) Kose et al. (2020)
RAD54 <sup>-/-</sup> mutant chicken DT40 cells		$\gamma$ H2AX Chromosomal aberration	125 $\mu$ M for 1 h 62.5, 125, and 250 $\mu$ M for 48 h		Lee S et al. (2013)
<i>S. Typhimurium</i> strains TA98, TA100		Ames test	4, 20, 100, and 500 $\mu$ g/plate $\pm$ 59	Negative	Fic et al. (2013)

BEAS-2B cells: human lung-derived epithelial cell line; BPAF: bisphenol AF; BPAF: bisphenol AP; BPF: bisphenol F; BPS: bisphenol S; Fpg: formamidopyrimidine DNA-glycosylase;  $\gamma$ H2AX: histone family member X; HepG2 cells: human hepatoma-derived cell line; Hu-PBMC: humanized peripheral blood mononuclear cell model; IC: inhibitory concentration; OGG1: 8-oxoguanine glycosylase; RAD54<sup>-/-</sup>: defective in RAD54 gene; RWPE-1 cells: human prostate-derived epithelial cell line; *S. typhimurium*: *Salmonella typhimurium*.